IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Arne Elof Brändström	
Serial No:	854, 739	Examiner: J. Fan
Filed:	April 21, 1986	Group Art Unit: 121
For:	NOVEL COMPOUNDS	

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

S I R: I, Hans Bundgaard, declare that:

1. My curriculum vitae is as follows:

Born November 8, 1944 in Denmark
Cand. pharm (M.Sc.) 1968 (Royal Danish School of Pharmacy)
Lic.pharm. (Ph.D.) 1973 (pharmaceutics)
Dr. pharm. 1977

Appointments

1969-1980: Associate Professor, Dept of Pharmaceutics
1981- : Professor of Pharmaceutical Chemistry,
Royal Danish School of Pharmacy

Professional activities

Member of the National Board for registration of drugs in Denmark (1977-1984).

Member of the Danish Medical Research Council (1980 -).

Member of the editorial board of the following scientific journals:

International Journal of Pharmaceutics
Pharmacy International
Journal of Clinical Hospital Pharmacy
Advanced drug delivery reviews

Editor of Arch. Pharm. Chem., Sci. Ed. (1973 -).

Editor of the following monographs:

Drug Design and Adverse Reactions, A. Benzon Symposium 10, Munksgaard, Copenhagen 1977.

Optimization of Drug Delivery. A. Benzon Symposium 17, Munksgaard, Copenhagen 1982.

Allergic Reactions to Drugs (Handbook of Experimental Pharmacology, vol. 63), Springer-Verlag, Berlin, 1983.

Design of Prodrugs, Elsevier Science Publishers, Amsterdam, 1985.

Research activities

Papers in international journals: about 180

Patents or patent applications:	5 filed since 1982 on various prodrugs for improved drug delivery (derivatives of metronidazole, allopurinol, pilocarpine, 5-fluorouracil and carboxylic acid agents)
Main research activity:	Development of novel drug delivery systems, in particular prodrugs.

A list of the latest papers is enclosed as Exhibit A.

2. I am familiar with the U.S. Patent Application Serial No. 854.739 of Brändström and with the official action dated October 24, 1986. I am also familiar with the U.S. Patent Application Serial No. 640.020 of Brändström, the parent application of the present application, and with the grandparent application Serial No. 586.107 of Brändström.

3. I have been asked by AB Hässle, the assignee of the present application, Serial No. 854.739, to give a statement on the stability of pharmaceutical agents with special reference to the stability of the free acid or base forms in comparison to salt forms. This statement follows below.

4. The chemical stability of drug substances in solid state or in solid pharmaceutical systems, e.g. tablets and capsules, is influenced by numerous factors.

5. The most important factors affecting stability is the presence of moisture, the storage temperature and exposure to light and oxygen (air). Furthermore, various pharmaceutical excipients such as buffer substances may affect the chemical decomposition. The chemical form of a drug, i.e. it be an acid or a salt, can also have a profounded influence on the chemical stability.

6. Drug substances may decompose by various mechanisms, the most common being hydrolysis and oxidation. For degradation of drugs in solid systems hydrolysis takes place in solution (e.g. formed by sorption of moisture as a thin film on the surface of the solid system) whereas oxidation can occur either in solution or in absence of moisture.

7. More detailed information on the chemical decomposition of drugs in the solid state and factors affecting the stability can be found in Exhibits B, C and D.

8. Concerning the chemical form of a drug (i.e., acid, base of salts thereof) there are no general rules for predicting the relative stability of e.g. acid and base addition salts thereof. As stated by Berge et al. in their review paper entitled "Pharmaceutical Salts", Exhibit E at page 1, Exhibit E.

"Salt-forming agents are often chosen empirically. Of the many salts synthesized, the preferred form is selected by pharmaceutical chemists primarily on a practical basis: cost of raw materials, ease of crystallization, and percent yield. Other basic considerations include stability, hydroscopicity, and flowability of the resulting bulk drug. Unfortunately, there is no reliable way of predicting the influence of a particular salt species on the behaviour of the parent compound. Furthermore, even after many salts of the same basic agent have been prepared, no efficient screening techniques exist to facilitate selection of the salt most likely to exhibit the desired pharmacokinetic, solubility and formulation profiles."

9. Depending on the route of degradation, a salt form of an acidic or basic drug may decrease of enhance the stability of the drug. Even different salts of the same drug substance may have a pronounced influence on the stability, e.g. due to differences in water solubility and hydroscopicity. Most often, however, a salt of an e.g. acidic drug appears to be more unstable than the neutral form. The principal reason for this is the fact that salts are often more water soluble than the neutral forms. Therefore, such salts have a greater ability to dissolve and react in an adsorbed moisture layer. Furthermore, for oxidation sensitive drugs, the ionized form (i.e., salt form) is generally much more reactive than the unionized (i.e., free acid) form.

10. Several specific examples corroborating this behaviour can be found in the pharmaceutical literature. A few examples are given in the following.

11. Aspirin is known to be much more unstable in salt form than as free acid. The drug is susceptible to hydrolytic decomposition. The lower stability of the salt forms is generally ascribed to the higher water solubility of salts like sodium or potassium salts and to the fact the the pH of solutions of the salts formed with an adsorbed moisture film is higher than pH of a solution of the free acid (Exhibits F and G). The hydrolysis of aspirin is accelerated with increasing pH.

12. Another example concerns ascorbic acid. Ritter et al (Exhibit H) have shown that the sodium salt of ascorbic acid is considerably more unstable than ascorbic acid when exposed to the same storage conditions.

13. A number of drugs sensitive to oxidation show a much higher stability as free acids compared to salt forms. This is thus the case for various phenols like salicylic acid, 5-aminosalicylic acid, epinephrine, apomorphine and morphine and sulfhydryl-containing molecules such as captopril (Exhibit I).

14. In conclusion, the stability of salt forms of acidic or basic drug substances can be markedly different from the stability of the parent substances. Generally, it is not possible to predict the influence of a particular salt form on the stability of the parent acidic or basic compound. In many cases, however, a more water soluble salt of an acidic drug is less stable than the parent free acid form.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent thereon.

Dated April 21, 1987

Hans Bundgaard

List of Exhibits

A. List of original publications

B. K.A. Connors, G.L. Amidon and V.J. Stella: "Chemical Stability of Pharmaceuticals", Second Edition, John Wiley & Sons, New York, 1986, Chapter 6

C. J.T. Carstensen: "Pharmaceutics of Solids and Solid Dosage Forms", John Wiley & Sons, New York, 1977, Chapter VII

D. J. Tingstad and J. Dudzinski: Preformulation Studies II: Stability of drug substances in solid pharmaceutical systems. J. Pharm, Sci. 62, 1973, 1856-1860

E. S.M. Berge, L.D. Bighley and D.C. Monkhouse: Pharmaceutical salts. J. Pharm. Sci. 66, 1977, 1-19

F. H.V. Maulding, M.A. Zoglio, F.E. Pigois and M. Wagner: Pharmaceutical heterogeneous systems IV: A kinetic approach to the stability screening of solid dosage forms containing aspirin. J. Pharm. Sci. 58, 1969, 1359-1362

G. C.A. Kelly: Determination of the decomposition of aspirin. J. Pharm. Sci. 59, 1970, 1053-1079

H. E. De Ritter, L. Magid, M. Osadca and S.H. Rubin: Effect of silica gel in stability and biological availability of ascorbic acid. J. Pharm. Sci. 59, 1970, 229-232

I. Exhibit B, Chapter 5

Exhibit A

Hans Bundgaard

List of original publications

1970

1. H.Bundgaard & K.Ilver: Kinetics of degradation of cloxacillin sodium in aqueous solution. Dan.Tidsskr.Farm. 44, 1970, 365-380.

1971

2. H.Bundgaard: Lægemiddel-latensering, princip og anvendelse. En oversigt. Dan.Tidsskr.Farm. 45, 1971, 73-86.
3. H.Bundgaard: Kinetic demonstration of a metastable intermediate in isomerization of penicillin to penicillenic acid in aqueous solution. J.Pharm.Sci. 60, 1971, 1273-1275.
4. H.Bundgaard: The lipophilic character of carboxy derivatives of penicillins, and its relation to their antibacterial activities. Dan.Tidsskr.Farm. 45, 1971, 283-288.
5. H.Bundgaard: Imidazole-catalyzed isomerization of penicillins into penicillenic acids. Tetrahedron Lett. 1971, 4613-4616.

1972

6. H.Bundgaard: Chemical aspect of penicillin allergy: imidazole-catalyzed isomerization of benzylpenicillin into benzylpenicillenic acid. Dan.Tidsskr.Farm. 46, 1972, 29-40.
7. H.Bundgaard: Chemical aspect of penicillin allergy: the reaction of penicillins with imidazole. Dan.Tidsskr.Farm. 46, 1972, 85-91.
8. H.Bundgaard: Penicillin allergy: imidazole-catalyzed formation of the penicilloyl determinant. J.Pharm.Pharmacol. 24, 1972, 985-987.

9. H.Bundgaard & K.Ilver: A new spectrophotometric method for the determination of penicillins. J.Pharm.Pharmacol. 24, 1972, 790-794.

10. H.Bundgaard: Penicillin-allergi, kemiske og farmaceutiske aspekter. Licentiatafhandling. København 1972, 79 sider.

1973

11. H.Bundgaard & C.Bundgaard: Spectrophotometric determination of microgram quantities of acetylsalicylic anhydride in acetylsalicylic acid. J.Pharm.Pharmacol. 25, 1973, 593-598.

12. H.Bundgaard: Intramolecular nucleophilic attack of an ureido group on the β-lactam carbonyl moiety of penicillins. Acta Pharm.Suec. 10, 1973, 309-316.

1974

13. H.Bundgaard: Acetylsalicylsalicylic acid: a potentially immunogenic impurity in acetylsalicylic acid. J.Pharm.Pharmacol. 26, 1974, 18-22.

14. H.Bundgaard: Spectrophotometric determination of ampicillin sodium in the presence of its degradation and polymerization products. J.Pharm.Pharmacol. 26, 1974, 385-392.

15. H.Bundgaard: Influence of an acetylsalicylic anhydride impurity on the rate of dissolution of acetylsalicylic acid. J.Pharm.Pharmacol. 26, 1974, 535-540.

16. H.Bundgaard & H.Angelo: Rate and mode of basic hydrolysis of anhydropenicillin. Tetrahedron Lett. 1974, 3001-3004.

1975

17. H.Bundgaard & A.L. de Weck: The role of amino-reactive impurities in acetylsalicylic acid allergy. Int.Arch.Allergy Appl.Immunol. 49, 1975, 119-124.

18. H.Bundgaard: Identification and quantitation of a 3-benzylidenephthalide contaminant of phenindione tablets and its characterization as a potentially immunogenic substance. Acta Pharm.Suec. 12, 1975, 333-348.

19. H.Bundgaard: Chemical studies related to cephalosporin allergy. I. Kinetics of aminolysis of cephalosporins and effect of C-3 substituents on β-lactam reactivity. Arch.Pharm.Chemi,Sci.Ed. 3, 1975, 94-123.

1976

20. H.Bundgaard: Hydrolysis and intramolecular aminolysis of cephalexin and cephaloglycin in aqueous solution. Arch.Pharm.Chemi,Sci.Ed. 4, 1976, 25-43.

21. H.Bundgaard: Polymerization of penicillins: kinetics and mechanism of di- and polymerization of ampicillin in aqueous solution. Acta Pharm.Suec. 13, 1976, 9-26.

22. H.Bundgaard: Chemical studies related to cephalosporin allergy. II. Competitive amine-catalyzed intra- and intermolecular aminolysis of cephalexin and cephaloglycin in aqueous solution. Acta Pharm.Suec. 13, 1976, 299-312.

23. H.Bundgaard: Kinetics of reaction of penicillins with amine and oxygen nucleophiles. A reference system for assessing chemical reactions involved in penicillin allergy. Arch.Pharm.Chemi,Sci.Ed. 4, 1976, 91-102.

24. H.Bundgaard: Colorimetric analysis of immunogenic impurities in acetylsalicylic acid. J.Pharm.Pharmacol. 28, 1976, 544-547.

25. H.Bundgaard & C.Larsen: Intramolecular and intermolecular transformations of aspirin in non-hydroxylic solvents.
J.Pharm.Sci. 65, 1976, 776-778.

26. H.Bundgaard: High-pressure liquid chromatographic determination of immunogenic contaminants of acetylsalicylic acid preparations.
Arch.Pharm.Chemi,Sci.Ed. 4, 1976, 103-113.

27. H.Bundgaard: Chemical aspects of penicillin allergy: mechanism of imidazole-catalyzed penicilloylation.
J.Pharm.Pharmacol. 28, 1976, 725-728.

28. H.Bundgaard: Allergic reactions: a potential side-effect of pro-drugs.
Acta Pharm.Suec. 13 Suppl., 1976, 23-24.

1977

29. H.Bundgaard: Polymerization of penicillins. II. Kinetics and mechanism of dimerization and self-catalyzed hydrolysis of amoxycillin in aqueous solution.
Acta Pharm.Suec. 14, 1977, 47-66.

30. H.Bundgaard: Polymerization of penicillins. III. Structural effects influencing rate of dimerization of aminopenicillins in aqueous solution.
Acta Pharm.Suec. 14, 1977, 67-80.

31. H.Bundgaard & C.Larsen: Polymerization of penicillins. IV. Separation, isolation and characterization of ampicillin polymers formed in aqueous solution.
J.Chromatogr. 132, 1977, 51-59.

32. C.Larsen & H.Bundgaard: A sensitive spectrophotometric method for determination of mecillinam or pivmecillinam in the presence of degradation products.
Arch.Pharm.Chemi,Sci.Ed. 5, 1977, 1-7.

33. H.Bundgaard: Allergic reactions to drugs mediated by chemically reactive impurities or degradation products. In H.Bundgaard, P.Juul & H.Kofod (eds.): Drug Design and Adverse Reactions. Alfred Benzon Symposium X, Copenhagen 1976. Munksgaard, Copenhagen 1977, p.165-183.

34. H.Bundgaard: Aminolysis of the 6β-amidinopenicillanic acid mecillinam. Imidazolone formation and intramolecular participation of the amidino side-chain. Acta Pharm.Suec. 14, 1977, 267-278.

35. H.Bundgaard: Penicillin allergy: kinetics of penicilloylation of serum albumins by various penicillins. Acta Pharm.Suec. 14, 1977, 391-402.

36. C.Larsen & H.Bundgaard: Kinetics and mechanism of degradation of mecillinam in aqueous solution. Arch.Pharm.Chemi, Sci.Ed. 5, 1977, 66-86.

37. H.Bundgaard: 4-Hydroxyphenylbutazone: a potentially immunogenic contaminant of phenylbutazone preparations. Arch.Pharm.Chemi, Sci.Ed. 5, 1977, 87-96.

38. H.Bundgaard: Quantitative determination of amino-penicillins in the presence of their degradation and polymerization products. Arch.Pharm.Chemi, Sci.Ed. 5, 1977, 141-148.

39. H.Bundgaard: Isolation and characterization of cephalexin degradation products formed in neutral aqueous solution. Arch.Pharm.Chemi, Sci.Ed. 5, 1977, 149-155.

40. C.Larsen & H.Bundgaard: Polymerization of penicillins. VI. Time-course of formation of antigenic di- and polymerization products in aqueous ampicillin sodium solutions. Arch.Pharm.Chemi, Sci.Ed. 5, 1977, 201-209.

1978

41. H.Bundgaard: "Lægemiddelallergi - kemiske og farmaceutiske aspekter". Disputats, 249 sider, København 1978.

42. C.Larsen & H.Bundgaard: Polymerization of penicillins. V. Separation, identification and quantitative determination of antigenic polymerization products in ampicillin sodium preparations by high-pressure liquid chromatography.
J.Chromatogr. 147, 1978, 143-150.

43. H.Bundgaard & C.Larsen: Kinetics and mechanism of the sucrose-accelerated degradation of penicillins in aqueous solution.
Int.J.Pharm. 1, 1978, 95-104.

44. C.Larsen & H.Bundgaard: Penicilloyl ester intermediates in glucose- and fructose-accelerated degradation of benzylpenicillin in aqueous solution.
Arch.Pharm.Chemi,Sci.Ed. 6, 1978, 33-40.

45. H.Bundgaard: Allergiske bivirkninger af acetylsalicylsyre. Immunkemisk baggrund og muligheder for nedsættelse af bivirkningsfrekvensen.
Arch.Pharm.Chemi 85, 1978, 245-260.

46. H.Bundgaard: Impurities as a factor in therapeutic equivalence of drugs: Analysis of ampicillin formulations for antigenic polymerization products.
Arch.Pharm.Chemi,Sci.Ed. 6, 1978, 63-68.

47. H.Bundgaard: A new stability-indicating spectrophotometric assay for 21-hydroxy corticosteroids and a kinetic assay for 21-dehydro corticosteroid impurities.
Arch.Pharm.Chemi,Sci.Ed. 6, 1978, 127-139.

48. B. Vej-Hansen, H.Bundgaard & B.Kreilgård: Kinetics of degradation of oxytetracycline in aqueous solution. Arch.Pharm.Chemi, Sci.Ed. 6, 1978, 151-163.

49. H. Bundgaard & C.Larsen: Kinetics and mechanism of reaction of benzylpenicillin and ampicillin with carbohydrates and polyhydric alcohols in aqueous solution. Arch.Pharm.Chemi, Sci.Ed. 6, 1978, 184-200.

50. B. Vej-Hansen & H.Bundgård: Kinetic study of factors affecting the stability of tetracycline in aqueous solution.
Arch.Pharm.Chemi, Sci.Ed. 6, 1978, 201-214.

51. H. Bundgaard: Increased specificity of the spectrophotometric imidazole-assay for ampicillin.
Arch.Pharm.Chemi, Sci.Ed. 6, 1978, 215-222.

52. H. Bundgaard: High-performance liquid chromatographic determination of phenylbutazone and its major degradation products in pharmaceuticals.
Arch.Pharm.Chemi, Sci.Ed. 6, 1978, 223-230.

53. H. Bundgaard, A.Bagger Hansen & C.Larsen: Pro-drugs as drug delivery systems. I. Esters of malonuric acids as novel pro-drug candidates of barbituric acids.
Arch.Pharm.Chemi, Sci.Ed. 6, 1978, 231-240.

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54. H. Bundgaard: Bioreversibel derivatisering af farmaka. Princip og anvendelsesmuligheder med henblik på forbedring af lægemidlers terapeutiske effekt.
Arch.Pharm.Chemi 86, 1979, 1-39.

55. H. Bundgaard & J.Hansen: A new stability-indicating spectrophotometric method for the determination of corticosteroids in aqueous media.
Arch.Pharm.Chemi, Sci.Ed. 7, 1979, 19-32.

(18) A. Brändström, P. Lindberg, B. Wallmark, Patent appl. EP 171372.

(19) K. Ankner, A. Brändström, P. Lindberg, P. Nordberg, B. Wallmark, Patent appl. EP 181846.

(20) P. Lorentzon, B. Eklundh, A. Brändström, B. Wallmark, Biochim. Biophys. Acta 817 (1985) 25-32.

(21) A. Brändström, Presented at the ASPET-ACS meeting in Boston, MA, August 18-22, 1985, Pharmacologist 27 (1985) 104.

(22) B. Wallmark, E. Carlsson, H. Larsson, A. Brändström, P. Lindberg, 3rd SCI-RSC Medicinal Chemistry Symposium in Cambridge, UK, September 15-18 (1985), Abstract S16. See also R. W. Lambert (ed.), Proceedings of the symposium, pp. 293-311.

(23) V. Figala, K. Klemm, B. Kohl et.al., 3rd SCO-RSC Medicinal Chemistry Symposium in Cambridge, UK, September 15-18, 1985, Abstract P20.

(24) P. Lindberg, P. Nordberg, XIth European Colloquium on Heterocyclic Chemistry in Ferrara, Italy, October 7-9, 1985, Abstract P65.

(25) A. Brändström, L. Tekenbergs-Hjelte, XIth European Colloquium on Heterocyclic Chemistry in Ferrara, Italy, October 7-9, 1985, Abstract P65.

(26) V. Figala, K. Klemm, B. Kohl, U. Krüger, G. Rainer, H. Schaefer, J. Senn-Bilfinger, E. Sturm, J. Chem. Soc., Chem. Comm. (1986) 125-129.

(27) P. Lindberg, P. Nordberg, T. Alminger, A. Brändström, B. Wallmark, J. Med. Chem. 29 (1986) 1327-1329.

(28) W. B. Im, J. C. Sih, D. P. Blakeman, J. P. McGrath, J. Biol. Chem. 260 (1985) 4591-4597.

(29) G. Rackur, M. Bickel, H.-W. Fehlhaber, A. Herling, V. Hitzel, H. J. Lang, M. Rösner, R. Weger, Biochem. Biophys. Res. Commun. 128 (1985) 477-484.

56. H. Bundgaard & C.Larsen: Pro-drugs as drug delivery systems. II. Open-ring ester derivatives as novel pro-drug candidates for trimethadione.
Arch.Pharm.Chemi, Sci.Ed. 7, 1979, 41-50.

57. B. Vej-Hansen & H.Bundgaard: Kinetics of degradation of rolitetracycline in aqueous solutions and reconstituted formulations.
Arch.Pharm.Chemi, Sci.Ed. 7, 1979, 65-77.

58. H. Bundgaard & C.Larsen: Piperazinedione formation from reaction of ampicillin with carbohydrates and alcohols in aqueous solution.
Int.J.Pharm. 3, 1979, 1-11.

59. H. Bundgaard: A differential kinetic method for the simultaneous determination of ampicillin and its pro-drugs pivampicillin and bacampicillin.
Arch.Pharm.Chemi, Sci.Ed. 7, 1979, 81-94.

60. H. Bundgaard: Simultaneous analysis of carbenicillin and its pro-drugs carindacillin and carfecillin by a differential kinetic method.
Arch.Pharm.Chemi, Sci.Ed. 7, 1979, 95-106.

61. H. Bundgaard, A.Bagger Hansen & C.Larsen: Pro-drugs as drug delivery systems. III. Esters of malonuric acids as novel pro-drug types for barbituric acids.
Int. J. Pharm. 3, 1979, 341-353.

62. H. Bundgaard & C.Larsen: Pro-drugs as drug delivery systems. V. Cyclization of methyl esters of succinamic and glutaramic acids to the corresponding imides (phensuximide and glutethimide) in aqueous solution.
Acta Pharm. Suec. 16, 1979, 309-318.

63. C. Larsen & H. Bundgaard: Hydrolysis of phensuximide. Isolation of degradation products and development of a stability-specific HPLC assay.
Arch. Pharm. Chemi, Sci. Ed. 7, 1979, 119 -127.

64. J. Hansen & H. Bundgaard: Studies on the stability of corticosteroids. I. Kinetics of degradation of hydrocortisone in aqueous solution.
Arch. Pharm. Chemi, Sci. Ed. 7, 1979, 135-146.

65. M. Johansen & H. Bundgaard: Pro-drugs as drug delivery systems. VI. Kinetics and mechanism of the decomposition of N-hydroxymethylated amides and imides in aqueous solution and assessment of their suitability as possible pro-drugs.
Arch. Pharm. Chemi, Sci.Ed. 7, 1979, 175-192.

66. H. Bundgaard, A. Bagger Hansen & C. Larsen: Pro-drugs as drug delivery systems. VII. Rapid cyclization of methyl diethylthiomalonurate to thiobarbital in aqueous solution.
Arch. Pharm. Chemi, Sci.Ed. 7, 1979, 193-198.

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67. J. Hansen & H. Bundgaard: Studies on the stability of corticosteroids. II. Kinetics and mechanism of the acid-catalyzed degradation of corticosteroids.
Arch. Pharm. Chemi, Sci.Ed. 8, 1980, 5-14.

68. H. Bundgaard & M. Johansen: Pro-drugs as drug delivery systems. VIII. Bioreversible derivatization of hydantoins by N-hydroxymethylation.
Int. J. Pharm. 5, 1980, 67-77.

69. H. Bundgaard: Pro-drugs as drug delivery systems. IX. Reversible cyclization kinetics of 2-aminoacetamido-5-chlorobenzophenone to demethyldiazepam in aqueous solution.
Arch. Pharm. Chemi, Sci. Ed. 8, 1980, 15-28.

70. H. Bundgaard & M. Johansen: Pro-drugs as drug delivery systems. IV. N-Mannich bases as potential novel pro-drugs for amides, ureides, amines, and other NH-acidic compounds.
J. Pharm. Sci. 69, 1980, 44-46.

71. H. Bundgaard & M. Johansen: Pro-drugs as drug delivery systems. X. N-Mannich bases as novel pro-drug candidates for amides, imides, urea derivatives, amines and other NH-acidic compounds. Kinetics and mechanisms of decomposition and structure-reactivity relationships.
Arch. Pharm. Chemi, Sci.Ed. 8, 198o, 29-52.

72. H. Bundgaard: The possible implication of steroid-glyoxal degradation products in allergic reactions to corticosteroids.
Arch. Pharm. Chemi, Sci. Ed. 8, 1980, 83-90.

73. J. Hansen & H. Bundgaard: Studies on the stability of corticosteroids. III. Separation and quantitation of hydrocortisone and its degradation products hy high-performance liquid chromatography.
Arch. Pharm. Chemi, Sci. Ed. 8, 1980, 91-99.

74. C. Larsen & H. Bundgaard: Kinetics of the acid-catalyzed cyclization of diclofenac to an indolinone in aqueous solution.
Arch. Pharm. Chemi, Sci.Ed. 8, 198o, 1oo-1o8.

75. H. Bundgaard: Drug allergy: chemical and pharmaceutical aspects.
Pharmacy International 1, 198o, 1oo-1o4.

76. H. Bundgaard: Pharmaceutical aspects of penicillin allergy: polymerization of penicillins and reactions with carbohydrates.
J. Clin. Hosp. Pharm. 5, 198o, 73-96.

77. H. Bundgaard: A differential kinetic method for the determination of mecillinam in the presence of its hydrolysis and epimerization products.
Int. J. Pharm.5, 198o, 257-266.

78. H. Bundgaard:Acid-catalyzed hydrolysis of fosfomycin and its implication in oral absorption of the drug.
Int. J. Pharm. 6, 198o, 1-9.

79. H. Bundgaard, E. Falch & C. Larsen: Pro-drugs as drug delivery systems. XI. Preparation and characterization of a novel water-soluble pro-drug type for barbituric acids.
Int. J. Pharm. 6, 198o, 19-27.

80. M. Johansen & H. Bundgaard: Pro-drugs as drug delivery systems. XII. Solubility, dissolution and partitioning behaviour of N-Mannich bases and N-hydroxymethyl derivatives.
Arch. Pharm. Chemi, Sci.Ed. 8, 1980, 141-151.

81. H. Bundgaard & J. Hansen: On the stability-indicating properties of some spectrophotometric assays for corticosteroids.
Pharm. Weekbl., Sci.Ed. 2, 1980, 127-128.

82. M. Johansen & H. Bundgaard: Pro-drugs as drug delivery systems. XIII. Kinetics of decomposition of N-Mannich bases of salicylamide and assessment of their suitability as possible pro-drugs for amines.
Int. Pharm. J. 7, 1980, 119-127.

83. H. Bundgaard: Kinetics and mechanism of rearrangement of penicillin to penicillenic acid in acidic solution.
Arch. Pharm. Chemi, Sci. Ed. 8, 1980, 161-180.

84. H. Bundgaard & J. Hansen: Studies on the stability of corticosteroids. IV. Formation and degradation kinetics of 21-dehydrocorticosteroids, key intermediates in the oxidative decomposition of corticosteroids.
Arch. Pharm. Chemi, Sci. Ed. 8, 1980, 187-206.

85. H. Bundgaard & M. Johansen: Pro-drugs as drug delivery systems. XIV. Bioreversible derivatization of phenylbutazone by C_4-aminomethylation to effect enhanced solubility and dissolution rate.
Arch. Pharm. Chemi, Sci.Ed. 8, 1980, 207-214.

86. J. Hansen & H. Bundgaard: Studies on the stability of corticosteroids. V. The degradation pattern of hydrocortisone in aqueous solution.
Int. J. Pharm. 6, 1980, 307-319.

87. H. Bundgaard & M. Johansen: Pro-drugs as drug delivery systems. XV. Bioreversible derivatization of phenytoin, acetazolamide, chlorzoxazone and various other NH-acidic compounds by N-aminomethylation to effect enhanced dissolution rates.
Int. J. Pharm. 7, 1980, 129-136.

88. H. Bundgaard & C. Larsen: Pro-drugs as drug delivery systems. XVII. Esters of 4-hydroxybutyric acids as potential pro-drug types for γ-lactones.
Int. J. Pharm. 7, 1980, 169-176.

1981

89. H. Bundgaard & J. Hansen: Studies on the stability of corticosteroids. VI. Kinetics of the rearrangement of betamethasone-17-valerate to the 21-valerate ester in aqueous solution.
Int. J. Pharm. 7, 1981, 197-203.

90. J. Hansen & H. Bundgaard: Studies on the stability of corticosteroids. VII. The effect of the dielectric constant on the rate of degradation of hydrocortisone in alcohol-water mixtures.
Arch. Pharm. Chemi, Sci. Ed. 9, 1981, 55-60.

91. J. Hansen & H. Bundgaard: Selective spectrophotometric determination of corticosteroids in tablets.
Arch. Pharm. Chemi, Sci. Ed. 9, 1981, 34-39.

92. M. Johansen & H. Bundgaard: Decomposition of rolitetracycline and other N-Mannich bases and of N-hydroxymethyl derivatives in the presence of plasma.
Arch. Pharm. Chemi, Sci. Ed. 9, 1981, 40-42.

93. M. Johansen & H. Bundgaard: Pro-drugs as drug delivery systems. XVI. Novel water-soluble pro-drug types for chlorzoxazone by esterification of the N-hydroxymethyl derivative.
Arch. Pharm. Chemi, Sci. Ed. 9, 1981, 43-54.

94. H. Bundgaard & M. Johansen: Pro-drugs as drug delivery systems. XVIII. Bioreversible derivatization of allopurinol by N-aminomethylation to effect enhanced dissolution rates.
Acta Pharm. Suec. 18, 1981, 129-134.

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Exhibit A

DRAFT GUIDELINE FOR STABILITY STUDIES FOR HUMAN DRUGS AND BIOLOGICS

March 1984

Center for Drugs and Biologics
Office of Drug Research and Review and
Office of Biologic Research and Review
Office of Drug Standards
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

Table of Contents

I. DEFINITIONS

Accelerated Testing: Studies designed to increase the rate of chemical or physical degradation of a drug substance or drug product by using exaggerated storage conditions. The purpose is to determine kinetic parameters, if possible, and/or to predict the tentative expiration dating period. This term is often used synonymously with "stress testing."

Commitment: A signed statement by an applicant of an New Drug Application (NDA), Paper NDA, Abbreviated New Drug Application (ANDA), Form 5 or 6, or Biological Product License Application (PLA) to conduct (or complete) prescribed studies after approval of an application. A commitment to obtain data may be accepted in lieu of the data themselves, when available data do not cover the full expiration dating period for the specific product/container-closure system, but there are sufficient supporting data to predict a favorable outcome with a high degree of confidence, e.g., when a new drug application is approved with stability data available only from experimental or pilot lots (not production lots) or when a supplement is approved with data that do not cover the full expiration dating period. A commitment constitutes an agreement to:

- conduct or complete the desired studies,
- submit results as they become available, or as specified by Food and Drug Administration (FDA), and
- promptly withdraw from the market any lots that do not meet approved specifications, and must be reported to FDA under 21 CFR 310.300(b)(1).

Drug Product: As defined under 21 CFR 210.3(b)(4), "drug product" means a finished dosage form, e.g., tablet, capsule, solution, etc., that contains an active drug ingredient generally, but not necessarily, in association with inactive ingredients.

Expiration Date: The date placed on the immediate container label of a drug product that designates the date through which the product is expected to remain within specifications. If the expiration date includes only a month and year, it is expected that the product will meet specifications through the last day of the month.

Expiration Dating Period: The interval of time that a drug product is expected to remain within specifications as determined from stability studies on a limited number of batches of the product. The expiration dating period is used to establish the expiration date of individual batches.

Paper NDA: An unofficial term referring to an NDA duplicating a previously marketed post-1962 product where the evidence of safety and effectiveness has been taken primarily from the published

literature. From a chemistry viewpoint, the term has no implication, as the requirements are identical to any other NDA.

Primary Stability Data: Data on the drug product stored in the proposed container-closure system for marketing at temperatures specified on the label.

Random Sample: A selection of units chosen from a larger population of such units in such a way that the probability of inclusion of any given unit in the sample is defined. In a simple random sample each unit has equal chance of being included. This differs from a haphazard sample in that for haphazard samples the probability of inclusion cannot be calculated. Random samples are usually chosen with the aid of tables of random numbers found in many statistical texts.

Stability-Indicating Methodology: Analytical methods that will quantitatively measure the characteristic structural and chemical properties of each active ingredient of a dosage form and distinguish them from their degradation products so that the active-ingredient content can be measured.

Stability: The capacity of a drug to remain within specifications established to assure its identity, strength, quality, and purity.

Strength: A quantitative measure of active ingredient, as well as other ingredients requiring quantitation, such as alcohol and preservatives. Also, see 21 CFR 210.3(a)(16).

Stress Testing: See "Accelerated Testing," page 1.

Supportive Stability Data: Data, other than primary stability data, such as stability data on investigational formulations not proposed for marketing, accelerated studies on the bulk drug substance, literature data, references to other submissions on file with the agency with appropriate letters of authorization, accelerated studies on the proposed drug product for marketing, information regarding test results on containers, and other scientific rationale to support the recommended storage conditions in the labeling.

Tentative Expiration Dating Period: A provisional expiration dating period determined by projecting results from less than full term data (such as accelerated studies) using the drug product to be marketed in the proposed container-closure system.

II. INTRODUCTION

Throughout this guideline reference is made to various IND and NDA requirements and to documents such as amendments and supplements submitted to new drug applications. FDA regulations relating to IND's and NDA's are in process of revision. Any revisions of these regulations will be reflected in subsequent versions of the

guideline.

This guideline provides:

- recommendations for the design of stability studies to establish appropriate expiration dating period(s) and product storage requirements (Section III), and

- recommendations for submission of stability information and data to the Center for Drugs and Biologics (CDB) for investigational new drugs and biologics (Section IV), new drug applications (Section V), and product license applications (Section VI).

This guideline should not be interpreted as imposing mandatory requirements [21 CFR 10.90(b)]. It does, however, describe the data and information considered desirable and acceptable by the agency in demonstrating the stability of drug products. It is intended to provide a means of meeting the regulatory requirements as listed below:

- IND's	21 CFR 312.1[312.23(a)(7)]
- NDA's	21 CFR 314.1(c)[314.50]
- ANDA's	21 CFR 314.1(f)[314.55]
- Form 6	21 CFR 431.1[341.55]
- Form 5	21 CFR 431.17[314.50]
- PLA's	21 CFR 601.2 --
- Supplements	21 CFR 314.8[314.70]

NOTE: The numbers in brackets refer to the proposed revision of the NDA and IND regulations (47 FR 46622, October 19, 1982 and 48 FR 26720, June 9, 1983).

This guideline provides a means of developing expiration dating from at least three lots (from different batches) in order to assure a statistically acceptable level of confidence for the period proposed. It is important, however, to realize that the manufacturer has the responsibility to confirm estimated expiration dating periods by continual assessment of stability properties from future lots. Such continuing confirmation of the expiration dating period should be an important consideration in the manufacturer's stability program.

III. DESIGN AND INTERPRETATION OF STABILITY STUDIES

The proposed revision of the NDA regulations in Section 314.70(d)(47 FR 46622, October 19, 1982) would permit an extension of the expiration date based on full shelf-life data obtained from an approved protocol and filing of data in annual reports. Submission of a supplemental application for FDA approval would not be required. The proposed revision empnasizes the importance of properly designing a stability study, as only use of an approved

protocol would entitle the applicant to proceed without a supplemental application. The design of the protocol should consider the methodology for determining the stability of the bulk drug substance and drug product and the statistics relating to sampling and data analysis.

A. Methodology Considerations

It is essential that stability indicating methods be used throughout all stability studies. The methodology should be validated by the manufacturer (and the accuracy and precision established) and described in sufficient detail to permit validation by FDA laboratories [1].

B. Bulk Drug Substance Profile

Stability information on the bulk drug substance is of value in anticipating problems which may be encountered in the formulation and storage of drug products as well as in establishing storage conditions and an expiration dating period for the bulk drug substance. The stability of the bulk drug substance is also an important consideration when establishing the expiration date on a batch of drug product prepared from aged bulk drug substance.

Stability studies on the bulk drug substance are needed when adequate stability information is unavailable either from prior studies or from the literature [2]. A program for the stability testing of the bulk drug substance should include storage in open and closed containers at ambient temperature and under stressed conditions. Stress testing conditions ordinarily include variable temperature (e.g., 5°, 50°, 75° C), humidity where applicable, (e.g., 75 percent or greater), and exposure to electromagnetic radiation (e.g., 190-380nm, ultraviolet, and 380-780nm, visible) and to fluorescent light (e.g., 500-2000 footcandles) [3-6]. It is also suggested that the following conditions, frequently encountered in drug product formulations, be evaluated in studies on solutions or suspensions of bulk drug substances:

- acidic and alkaline pH,
- high oxygen and nitrogen atmospheres, and
- the presence of added substances such as chelating agents and stabilizers.

It is important to detect, isolate, and identify degradation products. Degradation products should be quantified and the reaction kinetics established, if possible.

C. Drug Products

Stability studies on samples from production-size batches of the finished dosage form in the market package stored at the temperature stated on the label are ordinarily required but are not usually adequate without other information (e.g., stress testing) for assignment of an expiration date. Stress testing of the drug product is frequently used to identify potential problems that may be encountered during storage and transportation and to provide an estimate of the expiration dating period. Other special studies may be of value for specific drugs and/or dosage forms (see III.C.7.a-m, below).

When designing stability studies, the following should be considered:

1. Container-Closure

Stability data should be developed for each type of immediate container and closure proposed for marketing the drug that differs in composition and/or design (e.g., wall thickness, torque, etc.), including child-resistant and tamper-resistant closures, regardless of similarities in cap liners. Physician's samples should also be included in the stability studies if their container-closure system is different from the market package. The possibility of interaction between drug and container-closure system and the introduction of leachables into drug formulations during storage should be assessed by sensitive quantitative procedures. This is necessary even if the containers and closures meet suitability tests to protect drug integrity such as those outlined in the United States Pharmacopeia (U.S.P.) for plastic containers and rubber or plastic closures.

For most solid dosage forms stability data need only be obtained for the smallest and the largest container-closure system to be marketed, providing that any intermediate size container-closure systems are of identical composition. Special attention should be given to all sizes of multiple-dose containers such as aerosols and parenterals (see separate entries).

Where package container sealant integrity is to be assessed, higher than 75 percent relative humidity may be appropriate to stress its adhesive properties at 37°C (e.g., blister units and strip packages).

2. Extreme Temperature Fluctuations

A study of the effects of temperature fluctuation as appropriate for the shipping and storage conditions of the products should be considered, i.e., the packaged drug

should be cycled through temperature conditions that simulate the fluctuation that may be encountered once the drug product is in distribution channels. For example, it is suggested that all liquid preparations--injections, solutions, suspensions, and semi-solid preparations (creams, ointments, and pastes)--be subjected to freezing temperatures for at least seven days, and these observations should be utilized for appropriate labeled storage conditions or warning statement(s).

3. Storage Temperatures

The actual storage temperatures (numerical) used during stability studies should be specified.

4. Effects of Opening and Closing Containers

The effect on stability brought about by opening and closing the container should be assessed and compared with the stability pattern developed from unopened-container studies. The effect of opening and closing the container is simulated by sampling from the same containers at all scheduled test periods for as long as the contents permit rather than sampling unopened containers at each test period.

5. Microbial Quality

Drug products containing preservatives to control microbial contamination should have the preservative content monitored at reasonable intervals throughout the projected expiration dating period of the product. This may be accomplished by performing microbial challenge tests (e.g., Antimicrobial Preservatives Effectiveness test of the U.S.P., which is applicable to unopened containers) and by performing chemical assays for the preservative. When the minimum quantity of preservative to achieve effective microbial control has been determined, chemical assays may be adequate providing periodic challenge tests are performed [7]. It is particularly important to consider the adequacy of the preservative system under conditions of use for multidose vials [8].

Those preparations requiring control of the microbial quality that do not contain preservatives should be tested at specific intervals throughout the projected expiration dating period according to the release specification for bioburden (e.g., Microbial Limits Tests of the U.S.P.), which includes a limit for total microbial count and for the absence of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella species. In addition, it is recommended that topical preparations be tested for the absence of Pseudomonas cepacia, Aspergillus

niger, and Candida albicans as well as any other topical pathogens that may be identified as potentially harmful. Simulated use tests on topical preparations packaged in jars and on ophthalmics are desirable.

6. Degradation Products

When degradation products are identified, the following information should be submitted:

- chemical structure,
- cross-reference any available information about biological effect and significance at the concentrations likely to be encountered,
- procedure for isolation and purification,
- mechanism of formation, including order of reaction (See III.D.1.c., below)
- physical and chemical properties,
- specifications and directions for testing for their presence at the levels or concentrations expected to be present, and
- indication of pharmacological action or inaction.

7. Design Considerations for Specific Dosage Forms

a. Tablets: A stability study should include tests for the following characteristics of the tablet: appearance, friability, hardness, color, odor, moisture, strength, and dissolution.

b. Capsules: A stability study should include tests for the following characteristics: strength, moisture, color, appearance, shape, brittleness, and dissolution.

c. Emulsions: The following characteristics should be examined at each sampling interval: appearance (such as phase separation and color), odor, pH, viscosity, and strength. It is recommended that a heating/cooling cycle be employed, e.g., between 4° and 45° C [9 and 10].

d. Oral Solutions and Suspensions: The following characteristics should be examined at each sampling period: appearance (precipitate, cloudiness), strength,

pH, color, odor, redispersibility (suspensions), and clarity (solutions). Liquids and suspensions should be stored both upright and inverted in order to determine whether contact of the drug with the closure system affects product integrity.

After storage, samples of suspensions should be prepared for assay in accord with the recommended labeling under "Directions for Use".

e. Oral Powders: Most oral powders are marketed for reconstitution prior to administration. The following characteristics of the powder should be examined at each sampling period: appearance, strength, color, odor, and moisture. The reconstituted product should be prepared in accord with the recommended labeling under "Directions for Use". Specific characteristics to be examined on the reconstituted material should include: appearance, pH, dispersibility, and strength throughout the recommended storage period.

f. Metered Dose Inhalation Aerosols: Characteristics that should be examined in a stability study for all container-closure sizes include the following: strength (mg/ml), delivered dose (mg/valve actuation), number of (metered) doses, color, clarity (solutions), particle size (suspensions), loss of propellant, pressure, and valve corrosion [11].

Because the container contents are under pressure, filled containers must be checked for loss in weight over the expiration dating period. For suspensions, aggregate (or solvate) formation may lead to clogged valves, or the delivery of a pharmacologically inactive dose. Corrosion of the metering valve or gasket deterioration may adversely affect the delivery of the correct amount of drug substance.

As the drug product is intended for use in the lungs, it is important to consider at least the initial release specifications for the microbial limits (CFU/gram formulation) of total aerobic count, Gram-negative rods, and coagulase-positive Staphylococcus.

g. Topical and Ophthalmic Preparations: Included in this broad category are ointments, creams, lotions, pastes, gels, solutions, and non-metered aerosols for application to the skin. For stability studies of topical ointments, creams, lotions, solutions, and gels the following characteristics should be examined for all sizes as appropriate to the dosage form: appearance (clarity, color, homogeneity), odor, pH,

resuspendibility (lotions), consistency, particle size, strength, and weight loss (plastic containers).

Ointments and creams in containers larger than 3.5 grams should be assayed by sampling at the surface, middle, and bottom of the container. In addition, tubes should be sampled at other sites, e.g., near the crimp.

Evaluation of non-metered aerosols should include the following: appearance, odor, strength, pressure, weight loss, net weight dispensed, delivery rate, and spray pattern.

Evaluation of ophthalmic ointments, solutions, and suspensions should include as appropriate to the dosage form: appearance, odor, consistency, pH, resuspendibility, particle size, homogeneity (suspensions, creams, and ointments), strength, and sterility.

h. Small Volume Parenterals: Small volume parenterals include an extremely wide range of preparations and container-closure types. Each should be included in the stability study. Evaluation of these products should include at least the following: strength, appearance, color, clarity (freedom from visible foreign matter), pH, and sterility (at reasonable intervals). Stability studies on powder products should demonstrate that the residual moisture content remains within acceptable limits and that the product is stable throughout the recommended storage period. The stability of reconstitued freeze-dried products should also be determined.

Parenterals (except ampules) should be stored both upright and inverted in order to determine whether contact of the drug with the closure system affects product integrity.

i. Large Volume Parenterals: Stability tests for LVP's are similar to those appropriate for small volume parenterals. All container-closure sizes should be studied. A minimum evaluation should include the following: strength, appearance, color, clarity, particulate matter (U.S.P. or equivalent), pH, volume (plastic containers), extractables (plastic containers), and sterility (at reasonable intervals).

Continued assurance of sterility for all sterile products may be assessed by a variety of means, including examination of the container-closure system, testing for preservatives (if present), or sterility testing (at reasonable intervals).

For terminally sterilized drug products a specification for maximum process parameters should be provided. Stability studies should evaluate and support the maximum release specification for process lethality (e.g., F_0, Mrads, etc.).

These products should be stored both upright and inverted in order to determine whether contact of the drug with the closure system affects product integrity.

j. Suppositories: Suppositories should be evaluated for strength, melting range, appearance, and dissolution. The effect of aging may also be observed from a hardening of the suppository and a polymorphic transformation of the drug substance; therefore, control and stability testing should include dissolution time at 37° C.

k. Drug Additive: For any drug that is intended for use as an additive to another drug, the possibility of incompatabilities exists. In such cases, the product labeled to be administered by addition to another drug (e.g., parenterals, aerosols) should be studied for stability and compatability in admixture with the other drug.

A suggested protocol should provide for tests to be conducted at 0-, 6- to 8-, and 24-hour intervals. These should include:

- assay of the drug and additive,
- pH (especially for unbuffered LVP's) color, clarity, and
- interaction with the container.

l. Intrauterine Devices and Vaginal Devices Regulated as Drugs: Stability testing for intrauterine devices (IUD's) should include the following tests: deflection of horizontal arms or other parts of the frame if it is not a T-shaped device (frame memory), tensile strength of the withdrawal string, and integrity of the package, i.e., seal strength of the pouch and sterility of the device.

If the device contains a drug reservoir from which drug diffuses through a controlled release membrane, it should be tested for total drug content, decomposition products, and in vitro drug release rate in addition to the above tests.

Vaginal devices such as a doughnut shaped silastic or other polymeric matrix containing a drug uniformly

dispersed throughout the matrix must be checked for in vitro drug release rate and extraneous extractable substances to establish stability and drug compatibility with the matrix.

m. Biological Products: In addition to other parameters described for specific dosage forms, it is required for biological products that potency be a measure of biological activity. Generally, the official potency test (21 CFR Parts 600-680) or the potency test described in the manufacturer's approved license application for a given product will be adequate for potency determination.

N.B! Solid dosage forms, e.g., tablets, capsules, suppositories, powders (oral and parenteral), should be assayed for concentration per unit dose and per unit weight, when possible. This will permit more accurate assessment of product stability by explaining data fluctuations caused by variations in filling, tableting, etc. during manufacture.

D. Statistical Considerations

Proposed changes in the NDA regulations (47 FR 46622, October 19, 1982) permit a drug sponsor to take certain actions on the basis of an approved stability study protocol, such as extending an expiration dating period based on full shelf-life data without prior approval of a supplemental application by including the change in the next annual report under 21 CFR 314.80(c)(4)(iv). For this reason, a stability study protocol must describe not only how the stability study is to be designed and carried out, but also the statistical methods to be used in analyzing the data. An acceptable approach is described in part 2, below. If the sponsor wishes to use an alternative statistical procedure, it must be described in the stability study protocol. Part 1 of this section describes specific design features of stability studies that are pertinent to the statistical analysis.

1. Design Considerations for Long-Term Studies Under Ambient Conditions (Non-Accelerated Data)

The design of a stability study is intended to establish, on the basis of testing a limited number of batches of a drug, an expiration dating period applicable to all future batches of the drug manufactured under similar circumstances. This approach assumes that inferences drawn from this small group of tested batches extends to all future batches. Tested batches must, therefore, be representative in all respects (e.g., formulation, container/closure system, manufacturing process, age of bulk material, etc.) of the population of all production batches of that drug and conform with all quality specifications.

The stability study should be able to identify sources of variability and to quantify variability of individual dosage units, container-closure systems, and batches, as well as variability inherent in the laboratory test methodology. The degree of variability affects the confidence one might have in the ability of a future lot to remain within specifications until its expiration date.

a. Batch Sampling Considerations: Ideally, the batches selected for stability studies should constitute a random sample from the population of production batches already produced. In practice, the batches tested to establish the expiration dating period are usually the first batches produced, and there is a possibility that future changes in the production process will result in the obsolescence of the initial stability study conclusions. For this reason, additional batches should be subjected to stability studies whenever a substantial change occurs in the production process or formulation.

For unit dosage forms, such as solid oral dosage forms, variability in test values may be attributed both to assay and to unit-to-unit differences. For characterizing the batch average as a function of storage time, composites may be tested rather than individual units. However, if it is desired to characterize the unit-to-unit variance, then individual units must of course be tested. Variability of test results on bulk solutions, which are considered homogeneous, may be attributed solely to assay variance.

A sufficient sample of the batches should be taken to adequately assess within batch and between batch variability and to test the hypothesis that a single expiration dating period for all batches is justifiable. At least three batches and preferably more should be tested to allow for some estimate of batch to batch variability.

Testing of a single batch does not permit assessment of batch-to-batch variability, and testing of two batches provides an unreliable estimate. Although it is true that more data (batches) result in a more precise estimate, economic considerations prevent unlimited collection of data. Thus, the recommendation that at least three batches be tested is a compromise between statistical and economic considerations.

b. Container-Closure and Drug Product Sampling Considerations: Selection of drug products from the batches chosen for inclusion in the stability study

should be carried out in such a manner as to ensure that the samples chosen are representative of the batch as a whole; that is, they should be sampled randomly. At least two containers should be chosen from each batch at each sampling time.

In deciding how to sample the individual dosage units for assay, consideration should be given to the possible variability associated with the product. As a rule, the sampling of dosage units from a given container should be done randomly, with each dosage unit having an equal chance to be included in the sample. In the case of large containers, it may be suspected that dosage units near the cap of a bottle may have different stability properties than dosage units located in other parts of the container. In this case, it may be desirable to sample dosage units from all parts of the container suspected of giving different stability results. (For dosage units sampled in this fashion the location within the container from which they were taken should be identified. This information should be included when presenting the results.)

c. Sampling Time Considerations: The sample times should be chosen so that degradation can be adequately characterized if it occurs, i.e., at sufficient frequency to determine with reasonable assurance the nature of the degradation curve. Usually, the relationship can be adequately represented by a linear, quadratic, or cubic function on an arithmetic or a logarithmic scale (Section III.C.6).

For predictably stable drugs, stability testing may be performed at three-month intervals during the first year, six-month intervals during the second, and yearly thereafter.

The degradation curve is estimated most precisely (in terms of the width of the confidence intervals about the estimated curve, as illustrated in Figure 1) around the average of the sampling times included in the study. For this reason, sampling more frequently around the end of the desired expiration dating period is encouraged, since this will increase the average sampling time toward the desired expiration dating period.

2. Data Analysis and Interpretation; Long-Term Studies

When establishing the expiration dating period for an individual batch, consideration is given to the observed pattern of degradation for the quantitative drug characteristic of interest (e.g. strength) and to the

precision by which it is estimated. An acceptable approach, for drug characteristics that are expected to decrease with time, is to determine the time at which the 95% one-side lower confidence limit (sometimes called the 95% lower confidence bound) for the mean degradation curve intersects the acceptable lower specification limit. For the case of a linear degradation curve, the 95% one-sided lower confidence limit is the one-sided analogue of the two-sided limits described in Snedecor and Cochran [12], page 153, section 6.11. In the example shown in Figure 1, an expiration dating period of four years would be granted. For drug characteristics expected to increase with time (e.g. there may be an upper limit on the amount of certain degradation products), the 95% one-sided upper confidence limit would be used.

For drug characteristics with both an upper and a lower specification limit, there may be special cases where it would be appropriate to use the two-sided 95% confidence limits. As an example, suppose the drug characteristic of interest was concentration of unchanged active ingredient for a solution. Chemical degradation of the active ingredient would decrease the concentration. On the other hand, evaporation of the solvent (possibly resulting from the closure) would increase the concentration. Since both possiblities must be allowed for, two-sided confidence limits would be appropriate. (Of course, in the above example, if both mechanisms were acting the concentration might, e.g., decrease initially and then increase. In this case, the degradation pattern would not be linear and more complicated statistical methods would be needed.)

If this approach is used, we may be 95% confident that the average drug characteristic (e.g., strength) of the dosage units in the batch is within specifications up to the end of the expiration dating period. If it is desired to ensure that the characteristics of a large proportion (e.g., 90%) of the individual dosage units are within specifications, different statistical methods are needed. See, e.g., Easterling [13].

If batch-to-batch variability is small, i.e., the relationship between strength and time is essentially the same from batch to batch, it would be advantageous to combine the data into one overall estimate. Combining the data should be supported by a preliminary test of batch similarity. The similarity of the degradation curves for each batch tested should be assessed by applying statistical tests of the equality of slopes and of zero time intercepts. The level of significance of the test should be chosen so that the decision to combine is only made if there is strong evidence in favor of combining. Bancroft [14] has

recommended a level of significance of 0.25 for preliminary statistical tests similar to this.

If the preliminary statistical test rejects the hypothesis of batch similarity because of unequal initial strengths, it may still be possible to establish that the slopes are parallel, and in this case the data may be combined for purposes of estimating the common slope. The individual expiration dating period for each batch may then be determined by taking into account the initial potency values. For example, if the degradation curve, assumed here to be linear, estimates that a product initially at 100% strength would be reduced to 90% potency in 5 years, a product with initial potency of only 96% would be estimated to have an expiration dating period of 3 years.

If data from several batches can be combined, it is advantageous to include as many batches as feasible, because confidence limits about the estimated slope or the estimated degradation curve will become narrower as the number of batches increases, usually resulting in a longer expiration dating period.

If it is inappropriate to combine data from several batches, the overall expiration date may depend on the minimum time a batch may be expected to remain within acceptable limits.

3. Precautions To Be Observed in Extrapolation Beyond the Actual Data Period

The statistical methods for determining an expiration period beyond the range of storage times actually observed are the same as for determining an expiration period within the observed range. However, the a priori correctness of the assumed pattern of degradation as a function of time is crucial in the case of extrapolation beyond the observed range.

When estimating an assumed degradation line or curve over the observed range of data, the data themselves provide a check on the correctness of the assumed relationship, and statistical methods may be available to test the goodness of fit of the data to the assumed degradation line or curve. No such internal check is available beyond the range of observed data.

As an example, suppose it has been assumed that the relationship between log strength and time is a straight line, but in fact the true relationship is a curve. It may be that over the range of the observed data, the true curve is close enough to a straight line so that no serious error is made by approximating the degradation relationship as a

straight line. However, between the last observed data points and the estimated expiration time, the true curve may diverge from a straight line enough to have an important effect on the estimated expiration time.

For extrapolation beyond the observed range to be valid, the assumed degradation relationship must continue to apply through the estimated expiration dating period. For this reason, an expiration dating period granted on the basis of extrapolation should always be verified by actual stability data up to the granted expiration time as soon as these data become available.

E. Alternate Protocol

If for any stated reason the approach proposed in these guidelines is not suitable for the new drug or biological product under development, a different stability study protocol should be designed by the sponsor during clinical phases of investigation (Section II.D). The sponsor should assure that the protocol is acceptable to the reviewers in the Center for Drugs and Biologics.

IV. INVESTIGATIONAL NEW DRUGS (IND's)

Studies conducted during development of a drug or biological product do not necessarily follow a rigid separation into Phases 1, 2, and 3, but the following is presented as a general IND development sequence that is intended to provide guidance for the development of product stability information during the investigational phases.

A. IND Phase 1

The stability characteristics of the bulk drug substance should be determined at the earliest possible time to support conditions of use of the bulk drug in toxicity studies (i.e. pre-IND studies, mixed with feed, etc.) and the stability of the drug substance in the initial formulations proposed for use in clinical pharmacological studies. This information should be included in the initial IND submission to FDA. Required stability information would be limited to that needed to demonstrate that the product would be stable for the duration of the investigation. If necessary, additional data may be submitted as they become available during the course of the clinical study.

B. IND Phase 2

Stability studies on the investigational formulations should be well underway by the end of Phase 2.

Drug or biological formulations developed during Phase 2 (as well as Phase 3) should be based upon the stability information developed from studies on bulk drug substance and/or on the stability of formulations prepared in experimental studies. The objectives of stability testing during Phases 1 and 2 are (a) to evaluate the stability of the investigational formulations used in the clinical trials and (b) to obtain the additional information needed to develop a final formulation (e.g., compatibility studies of potential interactive effects between the drug substance(s) and other components of the system). This information should be summarized and submitted to the IND when available.

C. IND Phase 3

The emphasis in stability testing during Phase 3 is on final formulations in their probable market packaging, on expiration dating, and on the study of degradation products when encountered. Studies to support the proposed expiration dating period should be completed, where possible, during Phase 3 for inclusion in the initial NDA, Form 5 or 6, or PLA for biological products.

V. NEW DRUG APPLICATIONS (NDA's)

A. Original Submissions

Ordinarily an original NDA submission should contain primary stability data and other suitable data (e.g., accelerated data) that, when subjected to appropriate statistical analysis (figure 1), support the proposed expiration date and the proposed storage conditions for labeling. This must be accompanied by the standard commitment to continue the stability study (See "Definitions"). As a condition for approval it is expected that samples of the first three production lots will be placed in the stability program for the full length of the expiration dating period for confirmation of the dating period assigned.

A full report on stability of the bulk drug substance should provide information outlined in Sections III.B and III.C.6 of the guidelines on general stability characteristics and degradation products.

Stability studies conducted for all formulations utilized during clinical investigations should be summarized as described in Sections III.A, III.C, III.D, and VII, of these guidelines.

The lots used for stability testing should comply fully with proposed specifications for the product in its market package. Studies to support an expiration dating period under defined storage conditions using several lots representative of the

product to be marketed should have been started as early as possible prior to the NDA.

It should be appreciated that a reviewer cannot refer to information or make any comparisons with data contained in another application. Stability data submitted in a Paper NDA must be complete within themselves.

B. Computation of Expiration Date

The computation of the expiration date of the new drug production lot should begin at the time of quality control release of that lot, and the date of such release should generally not exceed 30 days from the production date regardless of the packaging date.

If the production lot contains reprocessed material, the expiration date shall be computed from the production date of the oldest reprocessed lot contained in the new lot.

C. Abbreviated New Drug Applications (ANDA's)

In the case of drug products that have been approved for marketing under an ANDA, information such as stress testing, references to publications, and comparative stability data for the proposed drug product with that of the innovator's drug product (especially when utilized in bioavailability/ bioequivalence studies) are acceptable.

In the absence of sufficient room temperature data, stress testing will be accepted for granting a tentative expiration dating period, provided adequate information concerning stability of the drug substance has been submitted. The recommended stress testing conditions are:

- 37-40°C (or as appropriate for a particular drug product, e.g., suppositories), and

- 75 percent relative humidity (where appropriate).

Samples should be analyzed initially and at 1, 2, and 3 months. The parameters described under Section III.C should be considered when collecting stability data for various drug products. Available long-term stability data should be included and reported as outlined in section VII of the guideline.

If the results are satisfactory, a tentative expiration dating period of 12 to 18 months will be granted for drug products packaged in unit-dose containers. For drug products packaged in other container-closure systems, the tentative expiration dating period will be 24 months.

The submission should include a signed statement that:

1. Stability studies as outlined below will be performed.

   The first three production lots of the product should be placed on stability testing. Controlled room temperature stability testing should be done initially, at 3, 6, 9, 12, 18, and 24 months, and yearly thereafter until the desired expiration date of the product is reached. If more than one package size is marketed, the first three production lots of the smallest and the largest size (e.g., 3 lots of 100-tablet bottles and 3 lots of 500-tablet bottles) should be tested. Also, if more than one container/closure system is used for a particular size, stability data in each container/closure system should be submitted. Yearly thereafter, one production batch should be added to the stability program.

2. Results will be submitted as they become available.

3. Any lots that fall out of specifications will be withdrawn promptly from the market, and must be reported to FDA under 21 CFR 310.300(b)(1).

D. SUPPLEMENTS TO NEW DRUG APPLICATIONS

A Supplement may be classified under several categories as indicated below:

1. Changes in Formulation, Supplier, and Container-Closure

   A supplement that proposes a change in the drug's formulation, in the supplier of a drug substance, or in the container-closure system for a marketed drug product, will usually require the development of data to show that this change does not adversely affect stability. Usually, accelerated data demonstrating comparability with the previously approved drug product, plus the standard commitment to continue the stability study will suffice. For significant changes of products known to be relatively unstable, six months' data at the normal recommended storage temperature, as well as the data from accelerated conditions, may also be required.

   If the data give no reason to believe the proposed change will alter the stability of the product, the previously approved expiration dating period may be used.

2. Interchangeability of HDPE Containers

   A special case is the interchangeability of High Density Polyethylene (HDPE) containers for capsules and tablets that

meet standards and tests described in the U.S.P. In this instance, a supplement may be approved with no advance stability data, provided there is a commitment to do the stability testing.

3. New Manufacturing Facilities

For a change limited to a new manufacturing facility for the identical drug product using similar equipment, a commitment should be submitted to conduct stability studies on at least the first three production lots based on the approved protocol. Ordinarily, the already approved expiration dating period may be used under these circumstances.

4. Reprocessed Material

A supplement providing for the use of reprocessed material should include data to demonstrate that the reprocessed product has identity, strength, quality, and purity comparable to that approved in the NDA for the designated expiration dating period. Also, the standard commitment to continue the stability study should be submitted.

5. New Container Fabricator

When a new plastic container fabricator is proposed, with no change in materials or specifications, the applicant should have full specifications for the approved container to supply to the new fabricator. The new fabricator should submit manufacturing information to the applicant (or directly to the FDA), and should agree to inform the drug manufacturer immediately of any change in resin formulation. The applicant should provide the standard commitment to initiate stability studies on several production lots packaged in the container from the new fabricator. Under these circumstances, accelerated or preliminary stability data are not required, and the already approved expiration dating period may be used.

VI. PRODUCT LICENSE APPLICATION FOR BIOLOGICAL PRODUCTS (PLA)

A. General Guidelines for Biological Product Stability Studies

The active components of biological products are usually protein derived or other organic substances. Such substances are usually heat sensitive and require refrigeration or freezing to protect the potency of the product. Therefore, the methodologies and statistical analyses used for determining the stability characteristics and expiration dating period for drug products are not necessarily applicable to biological products.

Because of the complexity and variety of the composition of biological products, requirements for determining their stability may differ markedly among different types of products.

Documentation of biological product stability is required for all new biological products and when significant changes are made to the composition or to the container and closure systems for currently approved biologicals. The descriptions which follow offer guidance regarding when stability data is required for biologicals. All proposals and submissions related to biological product stability should either accompany the product license application in an original submission for licensure or be submitted as an amendment to an existing product license application. Each submission will be considered on an individual basis depending on the composition and characteristics of the product.

B. Original Submission

1. Studies Submitted with Application

Studies to support the expiration dating period of a biological product using at least 3 lots representative of the product to be marketed under defined storage conditions should be submitted at the time of license application filing. These lots should comply fully with proposed specifications for the product in its market package. Stability data from at least three lots is usually required for licensure approval.

2. Supportive Data

The approved expiration dating period of a biological product is normally based upon the interval of time for which data are available under the storage conditions stated in the labeling. Studies that address the stability of the product when in bulk storage (prior to filling) may be considered to support the expiration dating period of the finished dosage form. In addition, the effects of temperature fluctuations that may be encountered during shipment of the product should be determined.

3. Expiration Dating Period Granted with Commitment

In some instances, the stability data may not cover the full time period desired. It is possible to grant the desired expiration dating period provided that all data and information clearly support this conclusion and there is a sufficient lead time for development of data covering the desired expiration dating period. The standard commitment to continue the stability study must also be submitted.

C. Amendments

1. Change in Formulation and Container-Closure

An amendment to an approved PLA that proposes a change in the product's formulation, including, for example, the culture media for growing live organisms, or the container-closure system for the marketed product, will usually require the development of data to show that the proposed change has not adversely affected the stability of the product. In certain instances, accelerated storage data demonstrating comparability with the previously approved product plus the standard commitment will suffice. For certain biological products known to be relatively unstable this may require a minimum of six months' data at the normal recommended storage temperature together with data from accelerated conditions.

2. New Manufacturing Facility

For a change limited to a new manufacturing facility for the same licensed product using similar equipment, a commitment should be submitted to conduct stability studies on a minimum of the first three lots produced in the new facility. Ordinarily, the previously approved expiration dating period may be used under these circumstances.

3. Extension of Expiration Dating Period

An amendment requesting an extension in the expiration dating period should be accompanied by supporting updated stability data.

4. Reprocessed Material

When appropriate, an amendment providing for the use of reprocessed material should include data to assure that the reprocessed product will meet final product specifications. The standard commitment to subject any lots of the product made from reprocessed material to stability testing should accompany the amendment.

VII. CONTENT OF STABILITY REPORTS

It is suggested that stability reports include the following information and data to facilitate decisions concerning the stability proposals:

A. General Product Information

1. Name of drug and drug product or biological product.

2. Dosage form and strength.

3. Labeling and formulation. (The application should provide a table of specific formulations under study when more than one formulation has been studied.)

4. Composition, type, and size of container-closure.

B. Specifications and Test Methodology Information

1. Physical, chemical, and microbiological characteristics and prior submission specifications (or specific references to NDA or USP).

2. Test methodology used (or specific reference to NDA, prior submissions, or USP) for each sample tested.

3. Information on accuracy, precision, and suitability of the methodology (cited by reference to appropriate sections).

4. For biological products, a description of the potency test(s) for measuring biological activity, including specifications for potency determination.

C. Study Design and Study Conditions

1. Description of the sampling plan, including:

   a. Batches and number selected,
   b. Containers and number selected,
   c. Number of dosage units selected and whether tests were conducted on individual units or composites of individual units,
   d. Sampling times, and
   e. Testing of drug or biological products for reconstitution at the time of dispensing (as directed on the labeling) as well as after they are reconstituted.

2. Expected duration of the study.

3. Conditions of storage of the product under study (temperature, humidity, light).

D. Stability Data/Information

1. Lot number (research, pilot, production) and associated manufacturing date.

2. For antibiotic dosage forms, the age of the bulk active drug substance(s) used in manufacturing the lot.

3. Analytical data and source of each data point, e.g., lot, container, composite, etc. Pooled estimates may be submitted if individual data points are provided.

4. Relevant information on previous formulations or container-closure systems should be included (or referenced, if previously submitted).

E. Data Analysis and Conclusions

1. Documentation of appropriate statistical methods and formulas used in the analysis.

2. Evaluation of data, including calculations, statistical analysis, plots, or graphics.

3. Results of statistical tests used in arriving at microbiological potency estimates.

4. Proposed expiration dating period and its justification.

5. Release specifications (establishment of acceptable minimum potency at the time of initial release for full expiration dating period to be warranted).

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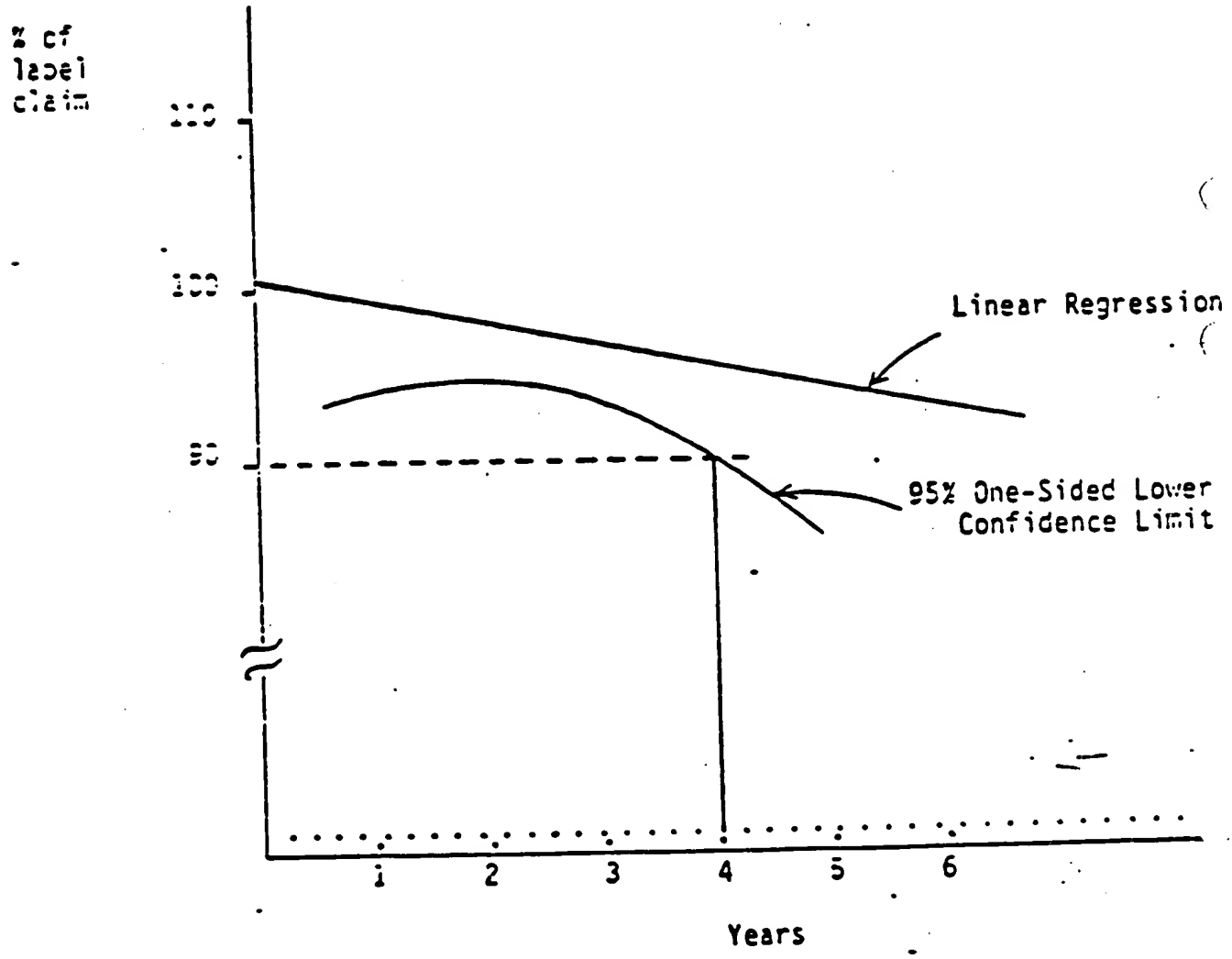
% of
label
claim
110
100
90
Linear Regression
95% One-Sided Lower
Confidence Limit
1
2
3
4
5
6
Years

Figure 1

Exhibit B

CHEMICAL STABILITY OF PHARMACEUTICALS

A HANDBOOK FOR PHARMACISTS

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A WILEY-INTERSCIENCE PUBLICATION

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Assumption: E_a for the drug decomposition is constant over the entire temperature range, that is, from the highest temperature experimentally studied to the extrapolation temperature (usually 25°).

Criterion: E_a = 10 kcal/mol and 20 kcal/mol provide reasonable and conservative limits for the temperature dependence of the reaction.

Criterion: The shelf-life is the time for product content to decrease to 90% of initial value or label claim.

This assumption is subject to experimental verification, and the criteria can be altered by the formulator to suit the requirements of a particular problem. The slopes of the lines in Figure 6.1 (which is essentially an Arrhenius plot) are determined by the chosen E_a values. The vertical displacement of the lines is controlled by the definition of shelf-life. Thus if more than a 2-yr goal estimate were desired, lines parallel to each of the curves in Figure 6.1 could be drawn that would meet the room-temperature abscissa at 36, 48, or 60 mo. Then a whole new plan of action could be generated analogous to the one described here.

C. STABILITY PROTOCOL

1. General Considerations

Although there is extensive variation in the stability-assessment programs within the pharmaceutical industry, we give some general considerations and an example of a stability protocol.

The main variables to be considered in a stability program are temperature, light, and moisture. In addition, container properties, preservative (microbial) stability, and physical characteristics (color, hardness, etc.) are part of many programs. The effect of light is usually studied in a light cabinet using clear and amber bottles, the effect of moisture by varying the relative humidity, and the effect of temperature in constant temperature cabinets.

A typical program might be as shown in Table 6.III where an X indicates that a sample is to be taken and

assayed at that time. Samples may be also stored at high humidity (>65%), a cycling test may be included, for example, 1 day at 10°C followed by 1 day at 30°C, and so on, and an actual shipping test, in which the product is shipped to a warehouse for a period of time and then returned for assay, may be included. Although the expiration date should be based on room temperature (or a specified temperature) data, the high-temperature data are often used to make preliminary estimates of the expiration date as discussed in the preceding section.

TABLE 6.III. Typical Stability-sampling Program

	Sample time (mo)											
t(°C)	0	1	2	3	4	6	12	18	24	36	48	60
4°							X		X			
25°	X	X		X		X	X	X	X	X	X	X
37°		X	X	X	X	X			X			
50°		X	X	X	X							

The protocol discussed here is typical for new product formulations. In many cases a more limited program could be used when, for example, minor formulation changes are made or a new container is to be introduced.

2. Experimental Designs

During the development of formulations, several formula variables are studied. Examples of these are flavors, colors, antoxidants, surfactants, chelating agents, buffers, and pH values. Normally these factors are studied by varying them in two ways, that is, by changing their concentrations (or level, as in the case of pH), or by omitting them from one formula and including them in another. It is obvious that trying to study too many variables simultaneously results in the need to make a large number of formulations to

Exhibit B

Chemical Stability of Pharmaceuticals

A Handbook for Pharmacists

Second Edition

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To the memory of Lloyd Kennon

J.T. Carstensen "Pharmaceutics of Solids and Solid Dosage Forms" Wiley & Sons, 1977 Exhibit C

VII. Stability and Dating of Solid Dosage Forms

There shall be assurance of the stability of finished drug products. This stability shall be ... recorded and maintained in such a manner that the stability data may be utilized in establishing product expiration dates.

Federal Register, January 15, 1971, 133.13.

VII-1 KINETIC PRINCIPLES

Drugs in solid dosage forms decompose much more slowly (in most cases) than the same drugs in suspension or solution form. The reason for this is that hydrolysis is a predominant decomposition route, and obviously solution dosage forms constitute a system much more prone to hydrolysis because of the abundance of water. Secondly, the molecules in solids are fairly fixed in space positionwise, whereas in solution and suspension they are subject to random movement, so that the interaction possibilities are greater in solution (or suspension) than in solids. Regarding oxidation, dissolved oxygen in solution is readily available for reaction, whereas in the solid phase, only surface oxidation with gaseous oxygen occurs. Internal molecules are somewhat protected.

Assays of drugs in solid dosage forms are less precise than in solutions because of the heterogeneity of the dosage form. The problem also exists that the decomposition is not exactly the same in each dosage unit, so that the highly precise decomposition kinetics often encountered in solution systems will never be found in the case of solid dosage forms. Whereas

the kinetics in solution might at times be carried out through many half-lives and assigned fractional reaction orders, a solid dosage form is rarely studied beyond one half-life, and one can distinguish between first- or zero-order decomposition patterns only (with modifications, as shall be shown later):

zero order $$C = C_0 - k_0 t, \qquad \text{(VII-1)}$$

First order $$\ln[C/C_0] = -k_1 t, \qquad \text{(VII-2)}$$

or $$\ln C = \ln C_0 - k_1 t, \qquad \text{(VII-2A)}$$

where C is drug content; k is rate constant; and time is denoted t and is usually in months. If x is the fraction decomposed, then

$$\frac{C_0 - C}{C_0} = 1 - (C/C_0) = x, \quad \text{i.e.,} \quad \frac{C}{C_0} = 1 - x, \qquad \text{(VII-3)}$$

so that Eq. VII-2 can be written:

$$\ln(1 - x) = -k_1 t, \qquad \text{(VII-4)}$$

but for values of $x < 0.15$ (i.e., less than 15% decomposed),

$$\ln(1 - x) \sim -x, \qquad \text{(VII-5)}$$

so that Eq. VII-4 may be written:

$$-x = -k_1 t \quad \text{or} \quad x = k_1 t, \qquad \text{(VII-5)}$$

or inserting Eq. VII-3:

$$1 - \frac{C}{C_0} = k_1 t \quad \text{or} \quad C = C_0 - (C_0 k_1) t, \qquad \text{(VII-6)}$$

which is a pseudozero-order reaction.

It is seen in Section VII-2 that there are solid dosage forms that adhere to Eq. VII-1, where k_0 is a (concentration-independent) constant. Conversely, Eq. VII-6 describes a *pseudozero-order reaction of the first kind.* It is noted that the rate constant is a function of initial concentration when C is plotted against time (as opposed to Eq. VII-1). Pseudozero-order reactions of the first kind are only applicable when the potency is above 85% of the initial potency.

In the following, to be consistent we use *only* Eqs. VII-1 and VII-2A for stability plotting. In other words, we do *not* use such plotting parameters as percent retained. Although it is perfectly sound to do so, it can at times lead to erroneous procedures for excess determinations.

One reason that stability tests are conducted is to arrive at the so-called excess. If a tablet is claimed to contain d mg of drug per tablet (label

claim, *LC*), and if the drug (as is usually the case) is somewhat labile, then the amount of drug per tablet will depend on time, that is, there will not (except at one point in time) be *d* mg of drug in the tablet. Since there must be some interpretation of how far the potency can deviate from *d*, the U.S.P. (and the F.D.A.) have established guidelines for *specifications*, and in general the potency must not fall below 90% of the labeled claim. In order to compensate for the loss in potency one adds an excess, (*xs*) that is, *d* mg plus some small amount. There are upper limits for the excess. Most U.S.P. items may contain no more than 10% excess so that the drug content of the product during its time on the market (i.e., prior to its purchase by the consumer) would be *d* mg ± 10%. By adding an amount in excess of *d* to start with, the tablet will contain *d or more* mg of drug for a period of time, *M* months, denoted the *shelf life*.

The data in Table VII-1 dealing with vitamin A stability in a tablet (1, 2) exemplify first-order decomposition. Since one data point is below 85% of the initial potency, there can be no question of the data being pseudozero-order, and they would have to be either zero- or first-order. In the process it is then also desirable to determine the rate constant. If the data in line 2 of Table VII-1 are plotted as ordinate and line 1 as abscissa on semilogarithmic paper, then as shown in Fig. VII-1A, a straight line ensues. Conversely, as shown in Fig. VII-1B, plotting on linear paper shows curvature.

A rough screening of reaction orders is frequently carried out by such plotting. It is possible to get an estimate of the rate constant k_1 from a semilogarithmic plot by the relation $kt_{50} = 0.693$ (as employed in other applications, e.g., blood-level curves plotted semilogarithmically, as well as some dissolution-rate plots). However, one rarely studies solid-state decompositions to 50% (one half-life), and it is more practical to use the relation:

$$-kt_{90} = \ln 0.9 = -0.1054, \quad \text{i.e.,} \quad k = \frac{0.1054}{t_{90}}. \qquad \text{(VII-7)}$$

Table VII-1 Vitamin A Potency (in mg) Retained Versus Time

Months Stored	Vitamin A (*C*, mg)	ln *C*
7.5	94	4.543
15	89	4.489
30	79	4.369

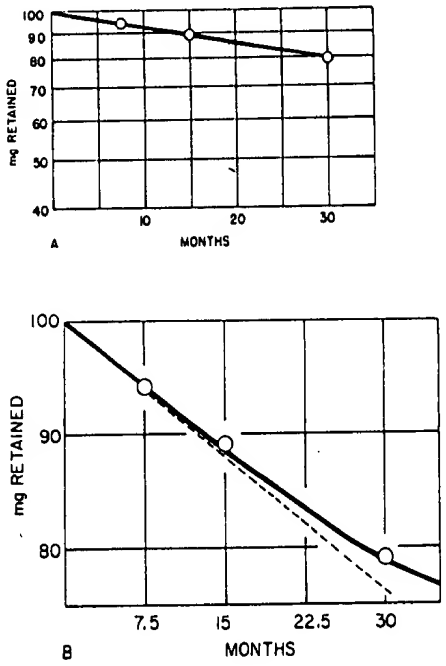


Fig. VII-1 **A:** First-order stability data plotted on semilogarithmic paper, and **B:** On linear graph paper.

The higher the losses, the more pronounced will be the difference between first- and zero-order data, and the more distinct the curvature of the data plotted by the incorrect order. If in the above case one extrapolated the data to longer times via a zero-order plot (and the data, as shown, are indeed first-order), then one obtains a higher loss rate than the true loss rate (Fig. VII-1B).

For exacting plotting, the data in a first-order reaction must be converted to their natural logarithms and these then plotted (or treated statistically). The k_1 value from Table VII-1 is:

$$\ln\left(\frac{79}{100}\right) = -0.236 = -k_1 30, \quad \text{so} \quad k_1 = 7.87 \cdot 10^{-3}\ \text{months}^{-1}.$$

The same value is obtained from the 7.5- and 15-month points. Note that the units in first-order decompositions in stability work usually are in months^{-1} (or year^{-1}).

In the case of a zero-order reaction, the rate constant k_0, (or the pseudozero-order rate constant) is expressed in mg/month (or mg/year). This is exemplified by the data on stability of ascorbic acid in multivitamin tablets shown in Table VII-2. DeRitter, Magid, Osadca and Rubin (3) showed that in such a product the potency of ascorbic acid dropped 6% in

Table VII-2 Stability of Ascorbic Acid in a 200-mg Tablet and 10-mg Tablet

Storage Time (Months)	200 mg Tablet 0% Excess, mg/Tablet	200 mg Tablet 18% Excess, mg/Tablet	10-mg Tablet mg/Tablet
0	200	236	10
6	194	230	9
12	188	224	8
36	164	200	6

one year in a "therapeutic" (i.e., 200-mg) formula. A 10-mg tablet in the same excipient base and of the same tablet weight, however, drops 20% in one year. If the reaction had been first order, then (Eq. VII-2) the percent retained would be the same, so the reaction must be zero order. It is seen from the last column that the rate is 1 mg/month, hence that the data are indeed zero order. To have a shelf life of $M = 36$ months, given the data in the second column, it would be necessary to add 36 mg to each figure. Doing this produces the data in the third column and as seen, the 36 mg (18%) excess gives a 36-month shelf life.

Example VII-1

A tablet contains 100 mg thiamine and a 10% excess. It retains 99% after 3 months, 98% after 6 months, and 96% after 12 months at 25°C. A 10-mg tablet of similar formula loses 10% of its potency in 3 months, 20% of its potency in 6 months, and 40% in 12 months. What is the order of reaction? What is the percent retained in the 100-mg tablet after 36 months? What excess should be used to give a shelf life of $M = 36$ months?

Answer VII-1

If the data from only the 100-mg tablet were available it would be difficult to establish whether the reaction were first- or zero-order. The data from the 10-mg tablet, however, establishes this as a zero-order case. After 3 months there will be $0.99 \cdot 110 = 108.9$ mg left, so that the loss rate is 1.1 mg/3 months = 0.367 mg/month. There will, therefore, be $36 \cdot 0.367 =$ 13.2 mg lost in 36 months. For a shelf life of $M = 36$ months it is necessary to use an excess of 13.2 mg (i.e., a 13.2% excess).

If the data for the 10-mg tablet had not been available in Example VII-1, it would not have been possible to establish whether the decomposition

Table VII-3 Stability of Thiamine (in mg) in a 100-mg Tablet

Months Stored at 25°C	Thiamine Present (100-mg Tablet)	Thiamine Present (10-mg Tablet)
3	109.2	9
6	107.9	8
13	105.8	6

was truly zero- or pseudofirst-order of the first kind, since all of the data are above 85% of the initial potency. From a practical point of view this does not matter as long as one knows by what order to treat the data, namely, zero order in this case. Data are never as "clean" as those tabulated above. More realistic data for a 100-mg tablet are shown in Table VII-3.

The data in Table VII-3 are not much different from those in Example VII-1 and are obviously zero order. The question is how to draw a line through these points. If the first point were used, the rate constant would equal 0.8/3 = 0.27 mg per month and if the second were used, the rate constant would be 2.1/6 = 0.35 mg per month. The correct way of drawing the line is shown in Fig. VII-2. The line is drawn "in between" the points; there are statistical methods for calculating such "least-squares fits" but they are beyond the scope of the material covered here. We employ here an approximate procedure primarily to illustrate the principles and definitions involved in stability work.

It would be natural to think that the stability equation for a solid dosage form that decomposed by zero order would be $C = [LC + xs] - k_0 t$. However, because of such factors as processing loss and variances due to processing and assay, C_0 is most often different from $LC + xs$. It is noted in Fig. VII-2 that the intercept, indeed, is *not* at 110 mg (which was the added amount).

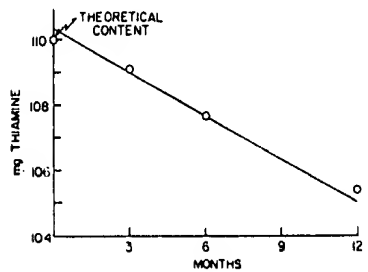


Fig. VII-2 Stability data of thiamine in a tablet formula.

Stability is considerably more complex than its kinetic aspects alone. Most compendial products have specification limits between 90 and 110% of the labeled amounts (label claim). Suppose as shown in Table VII-3 that a product has a label claim of 100 mg of drug; this means that the initial *assay* (performed by the compendial assay, if it is a compendial item) must now be within the range of 90–110 mg. During the time in commerce the *assay* must not fall below 90 mg. Compendial assays are usually based on an average of 20 tablets or capsules, and we shall use this as a definition of assay in the following.

In the data in Table VII-4 an excess of 5 mg has been used, that is, the tablets theoretically contain 105 mg of drug each. The initial assays reflect that there is a manufacturing loss, since the five assays average 104.6 mg/tablet with a standard deviation of $\sigma = 0.36$ mg. The stability data in Table VII-4 show that assays fall within 90–100% label claim after one year. On the average the assay after one year would be $104.6 - 4.4 = 100.2$ mg per tablet; this, as we shall see later, is approximate only. We can now draw the so-called stability line through the points (0, 104.6) and (1; 100.2). This line will cut 100% label claim (100 mg) after $4.6/4.4 = 1.05$ years (as can also be seen graphically). This is denoted the *shelf life*; it is noted that the shelf life is a function of the average loss rate, the initial loss, *and* the excess used.

The loss rate, however, could have been as great as $4.4 + (2 \cdot 2.05) = 8.5$ mg/year, in which case a 95% line through (0, 104.6) and (1, 96.1) is drawn. The 95% refers to 95% confidence (2σ limits). It can be seen graphically that this reaches the 90% label-claim limit (90 mg) at 20

Table VII-4 Zero-order Decomposition Data of a Product with a Theoretical Content of 105 mg of Drug*

Batch No.	mg/Tablet Initially	mg/Tablet After 1 Year at 25°C	k_0 (mg/year)	$\bar{k} - k_0 = \Delta$	Δ^2
102	105.3	100.9	4.4	0	0
109	104.1	97.3	6.8	2.4	5.76
111	104.4	102.8	1.6	2.8	7.84
112	104.7	101.3	3.4	1.0	1.00
113	104.6	98.7	5.9	1.5	2.25
Average:	104.6		$\bar{k} = 4.4$		$\Sigma\Delta^2 = 16.85$

*Standard deviation of $k = [16.85/4]^{1/2} = 2.05$ mg/year.

months, and this length of time is denoted the *expiration date* and the January or July closest prior to it is denoted the *label date*. Label dates must appear on labels by the new G.M.P. products.

It is obviously possible to improve this situation; if the initial data are analyzed it is noted that, since the standard deviation is 0.36 mg, the content of the tablet could be raised to $110 - (2 \cdot 0.36) = 109.28$ mg (= 109.28% label claim) with a 95% confidence that it would not supersede 110 mg initially. If more caution were exercised, then by adding $110 - (3 \cdot 0.36) = 108.92$ mg, there would be a 99.5% confidence (3σ limits) of this. Let us assume we choose this latter path; it can now be shown graphically that it would take 2.4 years to reach expiration. This can be arrived at arithmetically as well; when the tablets have suffered 18.92 mg loss, they will contain 90 mg; this will happen in $18.92/8.5 = 2.2$ year.

The approximate nature of the above treatment is obvious from the last calculation, because it is not clear whether one should use 108.92 mg or $108.92 - (2 \cdot 0.36) = 108.2$ as the initial figure. The lower limits determining expiration are thought of in Fig. VII-3 as being straight lines. In reality they are, as shown in Fig. VII-4, curved lines; The mathematics of these curved lines will be outlined in the following (16). In general a company desires to have a particular *expiration period* (36 months, 48 months) and hence adjusts the excess in the product to achieve the desired expiration period.

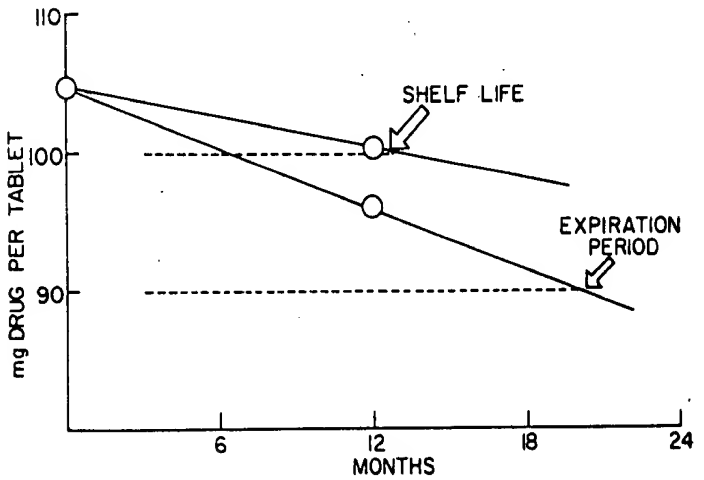


Fig. VII-3 Stability graphs showing the definition of *shelf life* by the least-squares-fit line and *95% expiration* by the lower (95% confidence) limit of the least-squares-fit line. This latter is *approximated* by a straight line.

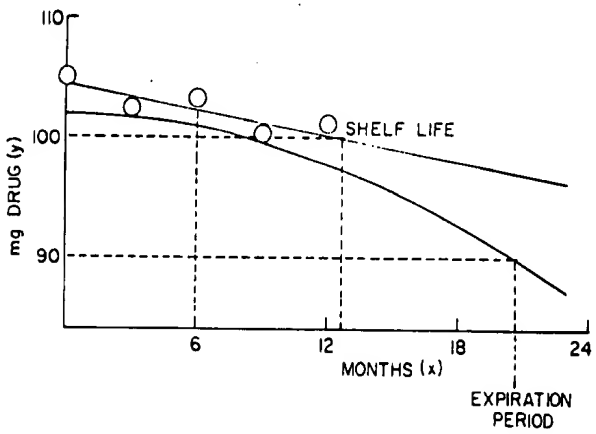


Fig. VII-4 Actual appearance of the lower (95% or other confidence) limits of the least-squares-fit line, showing shelf life and outdate. The narrowest point on the graph is for the average of the x values ($\bar{x}$). The lower limit is given by $\hat{y} - t_{n-2}s_{y.x}[(1 + (1/n) + \{(\bar{x} - \bar{x})^2/(\Sigma(x - \bar{x})^2\}]^{\frac{1}{2}}$ where $\bar{x}$ and $\hat{y}$ are the values on the least-squares-fit line. All points correspond to an average of 20 tablets, one determination per time point. $s^2_{y.x}$ is the sum of the squares of the distances of the points from the line after division by $(n - 2)$, where n is the number of points.

The general procedure for determining excesses is as follows: During phase III clinical-batch stability studies (or field trials of a product already marketed), the stability line is established. From this it is known whether the decomposition is zero (or pseudozero) order or first order.

In the *zero-order* case it is established that the statistically best stability line is:

$$C = C_0^* - k_0 t. \tag{VII-8}$$

As mentioned, C_0^* is not necessarily identical with $LC + xs$. This for $t = 36$ months (or other desired expiration period) gives a potency of

$$\overline{C_{36}} \pm \gamma_{0.9}. \tag{VII-9}$$

The interval is a 90% confidence interval, which means that there is a 95% probability of the assay being *above* $\overline{C_{36}} - \gamma_{0.9}$.

The excess that should be used is [90% label claim $- (\overline{C_{36}} - \gamma_{0.9})$] larger than the excess used in establishing the stability line. Note that if this calculates out to be negative, then the original excess used is too high. An excess of negative value (i.e., less than 0% excess) can however, not be used, and if the calculations indicate this, then no excess should be used to compensate for stability loss.

Example VII-2

A 100-mg aspirin product with 5% excess decomposes by a zero-order scheme. Data are collected for 2 years and the statistically best line determined to be $C = 104 - 0.5 \cdot t$, and the 90% confidence limits on the figure calculated at $t = 36$ months is $\overline{C_{36}} \pm 3$ mg. What excess should be used?

Answer VII-2

$\overline{C_{36}} = 104 - (0.5 \cdot 36) = 86$ mg ± 3 mg, that is, there is a 95% probability of the assay that will be performed after 36 months is above $86 - 3 = 83$ mg. We desire a 95% probability of it being above 90 mg (90% label claim). We hence add $90 - 83 = 7$ mg to the amount of aspirin (105 mg, not 104 mg), that is, 112 mg, so that a 12% excess is needed.

In the *first-order* case it is established during phase III or field trial, that the statistically best line is

$$\ln C = \ln C^* - k_1 t. \tag{VII-10}$$

This for $t = 36$ months (or other desired expiration period) gives a potency of

$$\overline{\ln C_{36}} \pm \gamma_{0.9}. \tag{VII-11}$$

The excess that should be used is so that ln [initial amount] is ln(90% label claim) $- [\overline{\ln C_{36}} - \gamma_{0.9}]$ higher than ln [amount initially added to the batches used in the stability-line determination].

Example VII-3

Vitamin A is studied for 2 years in a 100-mg tablet and the statistically best line (C being in mg and t in months) found to be $\ln C = 4.673 - 0.0048 \cdot t$. The tablet contains an 8% excess. When extrapolated to $t = 36$ months the calculated potency is found to be $\overline{\ln C_{36}} \pm 0.05$. What excess should be used?

Answer VII-3

$\overline{\ln C_{36}} = 4.673 - (0.0048 \cdot 36) = 4.5002 \pm 0.05$, in other words, there is a 95% probability that the 36-month assay is higher than given by ln(assay) = 4.4502. We desire there should be a 95% probability that the 36-month assay be above ln(90) = 4.4998, and we should hence increase $\ln C$ by $4.4998 - 4.4502 = 0.0496$.

The stability line is based on 108 mg added, that is, ln 108 = 4.682. We should, instead, use ln[added amount] = 4.682 + 0.0496 = 4.7316, that is, we should use 113.5 mg initially. The excess would hence be 13.5%.

It has been mentioned that the 95% line is not straight. Indeed is of the type (for a zero-order decomposition):

$$C = C^* - k_0 t - Ts\sqrt{\frac{1+n}{n} + [(t-\bar{t})^2/\Sigma\{(t_i-\bar{t})^2\}]} \qquad \text{(VII-12)}$$

T is here Student's t value for $n-2$ degrees of freedom, where n is the number of stability time points, t_i, used to establish the stability line. $\bar{t}$ is the average of the t_i values. $(n-2)\cdot s^2$ is the sum of the squares of the distances of the actual stability points (assays) from the stability line. The last term in the equation is synonymous with $\gamma_{0.9}$ and gives the 90% interval in which the performed assay (of 20 tablets) will lie after t months. A list of T values (student's t-values) is shown in Table VII-5.

Table VII-5 Values of Student's t (Denoted T In Text) For Various Degrees of Freedom ($n-2$)

n	$n-2$	$T_{0.1}$
3	1	6.31
4	2	2.92
5	3	2.35
6	4	2.13
7	5	2.02
8	6	1.94
9	7	1.90
10	8	1.86

Example VII-4

A 25-mg phenylephrine tablet shows the following stability line, C, being in mg and t being in months:

$$C = 26.5 - 0.125 \cdot t$$

A 5% excess is used in the preparation. What is $\overline{C_{36}}$? What excess would be needed to make the *shelf life* (not the expiration period) 36 months?

Answer VII-4

$C_{36} = 26.5 - (0.125 \cdot 36) = 22$ mg and, to have a 36-month shelf life the stability line should be at 25 mg after 36 months, so it is necessary to add $(25-22) = 3$ mg *more* of excess than the 5% (1.25 mg) used, in other words, a total of 4.25 mg or 17% excess is needed for this criterion.

Example VII-5

If the stability line for the phenylephrine product is given by

$$C = 26.5 - 0.125 \cdot t - 1.4 \cdot \sqrt{1.16 + [(t-5)^2/210]},$$

what is the lower 95% confidence limit of the concentration at 36 months, and what excess would be needed for a 36-month expiration period?

Answer VII-5

The lower 95% confidence limit would be given by:

$$22 - 1.4 \cdot \sqrt{1.16 + (31^2/210)} = 22 - 3.4 = 18.6 \text{ mg.}$$

Ninety percent label claim = 22.5, so it is necessary to add an additional $22.5 - 18.6 = 3.9$ mg to the 1.25-mg excess, that is, a total excess of $3.9 + 1.25 = 5.15$ mg, or about 20% excess.

Example VII-6

The data in Table VII-6 describe the assays found in thiamine tablets after various storage periods at room temperature. Assume the stability line calculated from these data to be: $C = 100 - 0.\bar{3} \cdot t$, where C is in units of mg/tablet and t is in units of months. What is the 95% confidence line?

Table VII-6 Thiamine Stability In Tablets

Time (t in months)	Thiamine (C in mg/tablet)
0	100
3	98.9
6	98.1
9	96.8
12	96.2

Answer VII-6

It is seen that $\bar{t} = (0+3+6+9+12)/5 = 6$ months. The values for $(6-t_i)$ and $(6-t_i)^2$ are tabulated in Table VII-7. The difference between the concentrations calculated from the stability line ($\bar{C}$) and the actual concentrations are shown, as are the squared numbers ($[\overline{C_i} - C_i]^2$).

$s^2 = (0+0.01+0.01+0.04+0.04)/(5-2) = 0.0\bar{3}$, in other words, $s = 0.18$. $T_{0.1}$ for $(n-2) = 3$ degrees of freedom. This is found from Table VII-5 to be 2.35, so $Ts = 0.18 \cdot 2.35 = 0.423$. $\Sigma(t_i - \bar{t})^2 = (36+9+0+9+36) = 90$, and $(1+n)/n = 6/5 = 1.2$, so the 95% confidence line is:

$$C = 100 - 0.\bar{3} \cdot t - 0.423 \cdot \sqrt{1.2 + [(t-6)^2/90]}.$$

Table VII-7 Thiamine Stability in Tablets

Time (t in months)	$(6-t)$	$(6-t)^2$	Thiamine (C in mg/tablet)	$\bar{C}$	$(\bar{C}-C)$	$(\bar{C}-C)^2$
0	6	36	100	100	0	0
3	3	9	98.9	99	0.1	0.01
6	0	0	98.1	98	−0.1	0.01
9	3	9	96.8	97	−0.2	0.04
12	6	36	96.2	96	0.2	0.04
	Total	90				0.1

VII-2 MOISTURE AND STABILITY

The single most important cause of loss of potency of a drug in a tablet (or capsule) is the presence of moisture (4–15). As shown in Fig. VII-5, the moisture will be present as surface moisture, which dissolves the drug to the extent of its solubility S. If the moisture is abundant (i.e., if there are considerably more moles of water than of drug), then the (pseudofirst-order) rate equation is:

$$\frac{dc'}{dt} = -kS \text{ mg cc}^{-1} \text{ months}^{-1}, \qquad \text{(VII-13)}$$

where c' is in concentration units (e.g., mg/cc), k is in months^{-1}, and the solubility, S, is in the same units as c'. If Eq. VII-13 is multiplied by the volume of water present, V(cc), then:

$$V\frac{dc'}{dt} = \frac{dC}{dt}\text{(mg/month)} = -kVS = -k_0'', \qquad \text{(VII-13A)}$$

which integrates to:

$$C = C_0 - k_0''t, \qquad \text{(VII-13A)}$$

which is a pseudozero-order reaction. To distinguish this from pseudozero-order reactions of the first kind (Eq. VII-6), these are denoted *pseudozero-order reactions of the second kind.* It is noted that when mg/tablet is plotted versus time on Cartesian (linear) graph paper, a straight line results with time as abscissa. The slope (pseudozero-order rate constant) is *independent of* C_0, as opposed to the reactions of the first kind. Example VI-1 is a case of a pseudozero-order reaction of the second kind.

Example VII-7

Librax capsules contain 2.5 mg of clidinium and 5 mg of chlordiazepoxide. After 2 years of storage there is 2.45 mg of clidinium and 4.75 mg of chlordiazepoxide left in an experimental batch and after 4 years of storage there is 2.4 mg clidinium and 4.5 mg of chlordiazepoxide left. The amount of excipient is 250 mg. Another experimental batch is made with the same amount of excipient but with 25 mg of clidinium and 50 mg of chlordiazepoxide. The amounts retained after 2 years are 24.5 mg and 49.25 mg and after 4 years, 24 mg and 49.50 mg. What reactions are taking place?

Answer VII-7

In the particular formulation the two drugs obviously decompose by zero order (since the loss rates are linear in time). In the case of clidinium, however, the loss rate at the low concentration is 0.025 mg/year and at the high concentration 0.25 mg/year, so it is a pseudozero-order reaction of the first kind. In the case of chlordiazepoxide the decomposition is pseudozero-order of the second kind because the reaction-rate constant (0.125 mg/year) is independent of chlordiazepoxide concentration. Note that the amount of excipient is the same, so that V is the same. A different amount of excipient would change V and this would affect the reaction order of the second kind (k_0'') because the V-term changes in Eq. VII-13A. It should be noted that the ***rate orders and rate constants are formulation dependent.***

Two points of caution should be made here: (a) the development leading to Eq. VII-13A assumed that there was an abundance of water (or that the water did not enter into reaction); in many cases, however, this is not so and (b) it might be tempting to assume that one could calculate k_0'' a priori; k could be obtained from solution kinetics, S from solubility

determinations, and V from moisture determination of the tablet. Such an approach can be used in model systems, as shown by Leeson and Mattocks(4), Kornblum and Sciarrone(8), and Carstensen and Pothisiri(1, 9). The complexity is, however, greater in a formulation (7–10) and some of the problems are as follows:

1. The pH dependence of k in hydrolyses is well established, but the pH of a "microenvironment" is not only difficult to determine but difficult to define.
2. Excipients in tablets contain water of hydration and other "bound" moisture and this is not available as part of the dissolution volume V, that is, only part of the amount of moisture determined by assay is "available."

There are, of course, cases where such a procedure is feasible. If the pseudofirst-order rate constant of the decomposition in solution is fairly pH independent in say, a range from pH 3 to 7 and, for an example case is $k = 7.72 \cdot 10^{-7}\ \text{sec}^{-1}$, and if it is determined that the solubility of the drug (also for this example assumed to be pH independent) is 25 mg/cc, then a calculation as described could be carried out. If the tablet weighed 500 mg and contained 10 mg of drug and the molecular weight of the drug were 300, and the tablet contained 5% of moisture but 3% of it was in the form of a hydrate and not "available," then the following would hold: $k = 60 \cdot 60 \cdot 24 \cdot 30 \cdot 7.72 \cdot 10^{-7} = 2\ \text{month}^{-1}$. The amount of "free" moisture would be $(0.05 - 0.03) \cdot 0.5 = 0.01$ g $= 0.01$ cc. This is 10/18 millimoles of water, which is considerably more than the 10/300 millimoles of the drug present. By Eq. VII-13A, the pseudozero-order rate constant in the tablet would be $2 \cdot 0.01 \cdot 25 = 0.5$ mg/month.

As mentioned, the starch bridge in a granule (Fig. VII-5) can be a source

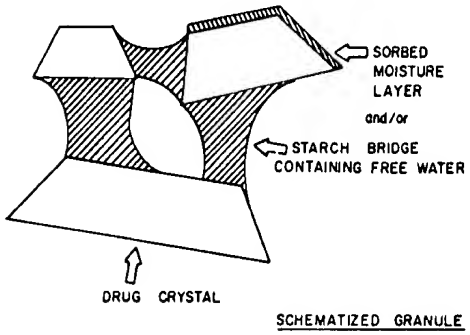


Fig. VII-5 Schematic figure of starch granule.

of available moisture. In the absence of other information one can *assume* that the water of hydration in a tablet is "bound."

Example VII-8

A tablet contains 250 mg lactose U.S.P. (monohydrate, with molecular weight 360), 3 mg (anhydrous) magnesium stearate, 20 mg of cornstarch (having 7% bound moisture), and 27 mg of (anhydrous) chlorprothixene. The tablet is found (by assay) to contain 15 mg of moisture. How much is free? (It is to be assumed that water of hydration is bound.)

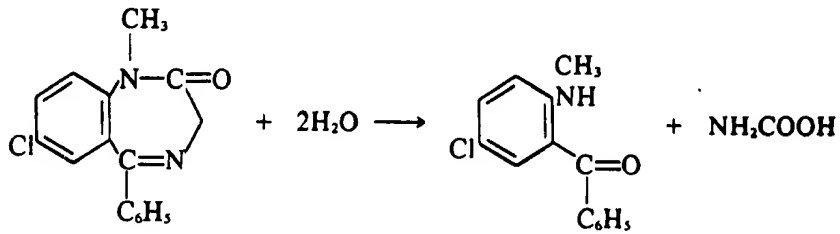
Answer VII-8

The lactose contains $250 \cdot (18/360) = 12.5$ mg of bound water; the cornstarch contains $20 \cdot 0.07 = 1.4$ mg of bound water; and the free (available) water amounts to $15 - 12.5 - 1.4 = 1.1$ mg of water per tablet.

Frequently the decomposition is a hydrolysis where water is used up as the tablet degrades, for example, thiamine contains an ethylene bridge, in other words, is R—CH_2—R′, and hydrolyzes to $RCH_2OH + R'H$, so that one mole of water is consumed per 1 mole of thiamine decomposed. If it is assumed that a tablet contains 0.5 mmole of drug and 0.02 g of water (i.e., 1.1 mmole), then there is sufficient water to decompose the entire drug content, and the solubility considerations outlined above hold. If, however, there were only 0.02 g of water (e.g., 0.2% in a 100-mg tablet), then there would be only 0.11 mmole of water present, and only that much drug can decompose by hydrolysis. This corresponds to $(0.11/0.5) \cdot 100 = 22\%$ decomposition.

Example VII-9

Diazepam decomposes by the following route in a tablet:



(VII-14)

(Actually, substituted carbostyril and substituted acridone are also formed, but they are disregarded here.)

A 300-mg tablet contains 5.6 mg of diazepam and 0.18% free moisture. If the above is the only decomposition route, what is the maximum decomposition?

Answer VII-9

Each tablet contains $5.6/280 = 0.02$ mmole of diazepam and $0.18 \cdot 300/(100 \cdot 18) = 0.03$ mmole of water which suffice to decompose $0.03/2 = 0.015$ mmole of diazepam. The amount of water is hence sufficient for hydrolysis of 75% of the diazepam. If hydrolysis alone occurred in a tablet (or capsule), and if the free moisture were not abundant, the potency would drop down to a level corresponding to the amount of water present, as shown in Fig. VII-6. Once the free water is used up, the

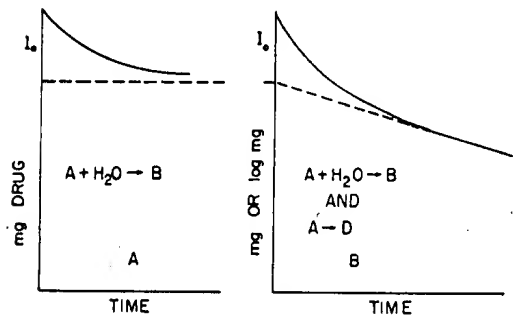


Fig. VII-6 Equilibrium conditions in tablet with limited amount of moisture when **A**: No other reaction takes place, and **B**: When a parallel reaction occurs.

degradation would stop and the potency would level off. Most often the hydrolysis is coupled with another (parallel) decomposition reaction, as shown in Fig. VII-6B, so the decomposition line will be a straight line (zero order) or log-linear curve (first order) intercepting the y axis below 100% (or log 100%). This intercept has a magnitude below 100% which is molecularly equivalent to the amount of free water present in the formula.

Example VII-10

A methyprylon tablet containing 100 mg of drug per tablet gives the following decomposition data at 25°C: (a) 10% lost after 6 months, (b) 13% lost after 12 months, and (c) 19% lost after 24 months. What would be an estimate of the free moisture present? What would be the excess needed for 3 years of shelf life? (The molecular weight of methyprylon is 183.)

Answer VII-10

From 6 to 12 months (i.e., a total of 6 months) the loss was 3 mg and from 12 to 24 months (i.e., a total of 12 months) the loss was 6 mg, so that the reaction is zero order with a rate constant of 0.5 mg per month. The intercept is hence $90 + 3 = 93$ mg, that is, 7 mg of drug $= 7/183 = 0.038$ mmole of methyprylon are lost by hydrolysis; this corresponds to $18 \cdot 0.038 = 0.68$ mg of water. The needed excess is found by noting that the potency after 36 months would be $93 - (36 \cdot 0.5) = 75$ mg, so that (Eq. VII-9) 25 mg (or 25%) excess is needed.

In general the stability report used by a company for excess determination and F.D.A. submission is based on room-temperature (25°C) stability data. Companies, however, also conduct *accelerated stability data*; they are carried out for the following reasons: (a) to establish excess in a previously nonexistent product, (b) to determine whether a decomposition is first or zero order, (c) as correlation test in stability *monitoring* of batches as they are being produced, and (d) to calculate stability of a product not stored at constant temperature. Accelerated studies are only valid when the decomposition path is the same at the higher temperature and the excipients are stable.

Whereas a chemical kineticist is interested in rate constants and activation energies, the stability pharmacist is interested in estimating room-temperature stability. Table VII-8 shows stability data that are plotted in Fig. VII-7 on semilogarithmic paper (first order).

A t_{90} value, as shown in Eq. VII-7, is the length of time it takes to "reach" 90% of the initial assay (i.e., 90% retained). The t_{90} values of the data in Table VII-8 are easily obtained from the graph in Fig. VII-7 and if these are plotted on semilogarithmic paper against reciprocal absolute temperature (Appendix A), the plot in Fig. VII-8 results. By extrapolating

Table VII-8 Accelerated Stability Data

Storage Period (in Months)	mg Retained at 55°C	mg Retained at 45°C	mg Retained at 37°C
0	100	100	100
0.5	90	—	—
1	79	94	96
1.5	73	—	—
2	65	90	96
3	—	82	93

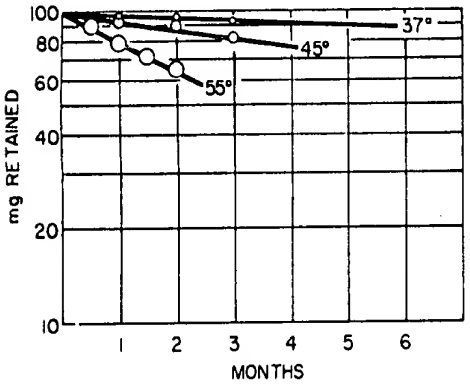


Fig. VII-7 First-order decomposition data at accelerated temperatures.

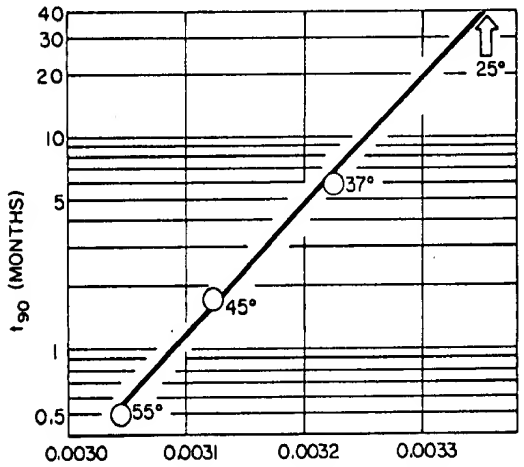


Fig. VII-8 Arrhenius plotting by t_{90} values. The extrapolated value at 25° occurs at $1/T = 0.00355$. The data at 55°C ($1/T = 0.003045$), 45°C ($1/T = 0.003145$), and 37°C ($1/T = 0.003225$) are from Fig. VII-7.

the t_{90} values to $1/T = 0.00335$ ($t = 25$°C), the t_{90} at room temperature is found to be 36 months (Fig. VII-8).

Example VII-11

In the data in Fig. VII-8 what excess should be used to ascertain 48 months of *shelf life.*

Answer VII-11

t_{90} at 25°C is seen from the graph to be 36 months. Using Eq. VII-7, $36k = 0.1054$, so $k = 0.00293$ month^{-1}. The reaction is first order (Fig. VII-7) so Eq. VII-2A gives

$$\ln C = \ln 100 - (0.00293 \cdot 48) = 4.6052 - 0.1406 = 4.4646,$$

that is, C after 48 months, with an initial assay at 100 mg would be exp (4.4646) = 86.9 mg.

For shelf life, C should be 100, so ln C should be 4.6052; in other words, the initial concentration C^* should have a natural logarithm 0.1406 higher than 4.6052, that is:

$$\ln C^* = 4.6052 + 0.1406 = 4.7458, \quad \text{so} \quad C^* = \exp(4.7458) = 115\%.$$

It is noted that if C is known at the desired expiration period, then the initial amount should be $C^* = LC(100/C)$ when the reaction is first order (but not when it is zero order). For instance, in Example VII-11,

$$C^* = 100\ LC/C_{at} = 100\ 100/86.9 = 115 \text{ mg}, \qquad \text{(VII-15)}$$

where C_{at} is the assay found in the stability study at the point in time which is the desired shelf life.

Although shelf lives can be estimated in accelerated studies, it is noted that *expiration dates cannot be estimated from accelerated data,* since the 95% confidence line cannot be constructed. It is possible to estimate the 90% confidence interval (95% by one-tailed estimation) for the rate constant at 25° from plots like Fig. VII-8; from this one can construct a lower 95% confidence line, but this is a straight line (not a curve as in Fig. VII-4) and hence is at best a very uncertain estimate of the actual 95% line.

From the product formulator's point of view (and that of the analytical chemist), there are a few more advantages to accelerated testing. It obviously can serve for screening purposes in preformulation studies (17), as a guide in formula selection in initial development, and for supporting evidence of physical equivalence of new/revised formula/procedure changes that would require an ammendment to a company's new drug application. Two further (frequently forgotten) reasons for accelerated studies are: (a) that they serve as early warning of unsuspected decomposition paths in early and middle development (phases I and II) and (b) since, in the early stages of product development, the assay methodology may not yet have been perfected, they present the analytical chemist with stressed systems having products that may interfere with what he thinks is a stability-indicating assay. This latter situation frequently leads to analytical discoveries during the development stage and may cause

analytical revisions during the stability program. Since it is important in this case to be able to "go back" and assay the initial sample with the new procedure, samples of importance are often "frozen" so that they can later be retrieved, should the situation call for it. The philosophy here is that a sample kept at −5° will suffer virtually no loss.

It should finally be mentioned, that the variation of drug content from tablet to tablet may vary with time also; this has been reported by Fusari(18), and is one of the reasons that, for instance, in field sampling there will be a larger standard deviation in old samples than attributable to batch-to-batch variation.

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PROBLEMS

VII-1 If there were no batch-to-batch variation and the data in Example VII-1 pertained, then the confidence lines around the stability line would vanish. In this case, what would be the needed excess for a shelf life of $M = 48$ months?

(Answer: 17.6 mg)

VII-2 If the data in Table VII-3 and Fig. VII-2 corresponded to a 100-mg tablet with no excess, then the best statistical line would be given by $y = 100.07 - 0.33x$, where y is mg of drug and x is months. Calculate the 95% confidence line, the shelf life, and the expiration period.

VII-3 If a 100-mg tablet has a 14% excess, how long will it (on the average) take to reach 88% of initial potency, assuming that the stability line is $C = 112 - 0.5t$? What is the shelf life?

(Answer: 26.9 months; 24 months)

VII-4 Reference is made to Example VII-9. The solubility of diazepam (molecular weight 269) in water is 0.01 mg/cc; if the reaction in Eq. VII-14 has a reaction rate of k_3[Diazepam][H_2O]^2, where $k_3 = 10$ molar^{-2} month^{-1}, what is the initial loss rate?

(Answer: 0.17 mg/month.)

VII-5 The least-squares fit line in Fig. VII-2 is $y = -0.333x + 110.07$, where y is mg retained and x is time in months. Exemplify (e.g., using the line $y = -0.4x + 110$) that another line gives a larger sum of squares ($\Sigma\Delta^2$). [Δ is the distance of a point from the given line; e.g., the third point (13 months) in Table VII-3 is 105.8 and the line point is $y = -0.333 \cdot 13 + 110.07 = 105.74$ so $\Delta = 105.74 - 105.8 = -0.06$).

VII-6 The following t_{90} data were found for a drug in a tablet product: t_{90} = 1 month at 55°, 6 months at 45°, and 16 months at 37°C. What is t_{90} at 25°C?

VII-7 DeRitter, Magid, Osadca, et al.(3) give the following amounts of ascorbic acid retained in a tablet containing 200 mg ascorbic acid: (a) after 1 month at 55°C, 91%, (b) after 3 months at 45°C, 84%, (c) after 6 months at 27°, 88%, and (d) after 12 months at 25°C, 94%. Plot these data (a) assuming they are zero order and (b) first order and calculate t_{90} at 15°C.

VII-8 The first-order presentation of the data in Table VII-1 (Fig. VII-1A) is $y_1 = 100\ 10^{-0.00336x}$, where x is time in months. The zero-order presentation (Fig. VII-1B, confined to 100% retained at time zero) has a least-squares fit of $y = -0.71x + 100$. Show that the first-order equation affords a better fit.

VII-9 What excesses would be used to ensure a 36-month shelf life if the data in Table VII-1 are plotted by (a) first order and (b) zero order? Use the least-squares-fit equations shown in Problem VII-8.

VII-10 Reference is made to the work by Fusari(16), in which Fig. 3 shows that individual assays are originally 104.9 with an (individual) $\sigma = 3.3\%$. After storage the individual tablet-to-tablet variation in assay has a standard deviation of 12.0%. The label claim is 400 μg of nitroglycerin per tablet. Assuming that the standard deviation of an average of 20 is the individual standard deviation divided by $[20]^{1/2}$ (i.e., omitting the student t effect), what is the standard deviation of the average of 20 after storage?

VII-11 A tablet contains 200 mg of a drug with a molecular weight of 312.5. The moisture content of the tablet is 0.5% (available) and the compression weight is 288 mg. The drug hydrolyzes rapidly, consuming 1 mole of water per 1 mole of drug. The drug by itself undergoes a (parallel) decomposition that is 12.5% after 2 years. What excess would be needed for a 2-year shelf life if the decomposition were (a) zero order and (b) first order?

VII-12 A product contains 250 mg of drug per tablet and shows 90.0% potency retained after 12 months, 82.0% retained after 24 months, and 74.0% retained after 36 months. (a) What is the order of the decomposition? (b) What excess would be needed to have a 36-month shelf life? (c) If the above data were 90%, 82%, and 74% (i.e., less precise), could the same conclusion have been drawn?

VII-13 A product is released or rejected based on an assay that is an average of a (composite) assay of 20 tablets. The single tablet-to-tablet variation has a standard deviation of 2.24%, and the batch-to-batch variation of the assay (of an average of 20) is 0.8%. If the specifications are 90–110% label claim, what is the largest excess that can be used? (Note that the standard deviation of an average of 20 is the single standard deviation divided by $\sqrt{20}$. *Variances* are additive.) The product decomposes at a rate of 0.3 mg per month. Estimate the best shelf life. (The tablet contains 100 mg of drug per tablet.)

(Answer: 108.4%, 28 months.)

VII-14 Given the following stability data, indicating the order of the reaction: (a) initial, 100 mg/capsule, 55°, (b) 1 month, 85 mg/capsule, 55°, and (c) after months, 72.5 mg/capsule. At room temperature, the assay after 6 months is 95 mg/capsule. What will it be after 3 years? (Calculate graphically.) What excess would you use to have a 3-year shelf life?

QUESTIONS

1. When the logarithm of potency (mg/tablet) is plotted against time and the data are first order, the slope of the line will be independent of initial concentration; (a) true or (b) false.
2. When potency (mg/tablet) is plotted against time and the data are pseudozero order of the first kind, the slope of the line will be independent of initial concentration; (a) true or (b) false.
3. A first-order reaction becomes indistinguishable (to within ±1%) from a zero-order reaction if: (a) the potency is above 85% label claim or (b) more than 85% of initial potency is retained.
4. A powder loses 20% of its original drug content in first-order fashion in three years. This would correspond to a recommended excess of: (a) 25%, (b) 20%, or (c) 33% if it were desired that the drug content be 100% label claim of the particular sample after 3 years. Assume initially a first-order decomposition.
5. A first-order decomposition is plotted on: (a) linear graph paper or (b) semilogarithmic graph paper to produce a straight line.
6. A zero-order decomposition is plotted on: (a) linear graph paper or (b) semilogarithmic graph paper to produce a straight line.
7. The moisture adsorption theory predicts: (a) first-order decomposition, (b) pseudozero-order decomposition of the first kind, or (c) pseudozero-order decomposition of the second kind.
8. A tablet contains 100 mg of drug initially, 96 mg after 6 months, and 94 mg after 9 months at room temperature. The decomposition is: (a) first order, (b) pseudozero order of the first kind, or (c) pseudozero order of the second kind, (d) either of the above, depending on further data.
9. Given that a tablet initially contains 100 mg, then 90 mg after 3 months, 80 mg after 6 months, and 70 mg after 9 months, is the decomposition: (a) first order, (b) zero order, or (c) possibly either (a) or (b), pending further data.
10. Given that a tablet initially contains 100 mg, contains 80 mg after 3 months, 64 mg after 6 months, and 51 mg after 9 months, the decomposition is: (a) first order, (b) zero order, or (c) possibly either (a) or (b), pending further data.

11. If the data in Question 10 were obtained at 55° and the data in Question 8 at room temperature, should the latter be plotted on: (a) semilogarithmic or (b) linear paper?

12. Two batches of the same product show stability data that level off at 90% of initial assay for batch No. 1 and 86% of initial assay for batch No. 2. These levels are reached at room temperature and accelerated temperatures as well. Is the decomposition: (a) caused by available moisture, (b) a thermal decomposition of the drug itself, or (c) a chemical equilibrium?

Exhibit D

viscosity were studied on Days 1, 2, 7, 14, 30, and 60. Day 1 corresponded to the day the emulsions were prepared.

When stored at high comparable temperatures for the study period, emulsions stabilized by a quaternary amine (cetylpyridinium chloride) were more stable than those stabilized by a surfactant precursor amine (2-amino-2-methyl-1,3-propanediol) and linoleic acid. All emulsions stored at room temperature (25°) and at 0° were stable.

Photomicrographs were used to study droplet size change. High surfactant concentrations retarded droplet size growth. A sudden increase of droplet size preceded demulsification, as was very evident at high temperatures.

A rapid decrease in viscosity preceded demulsification. At high temperatures, the cetylpyridinium chloride-stabilized emulsions formed loose gels and exhibited erratic readings.

Thixotropy was demonstrated as the emulsions were allowed to age at least 2–4 weeks. It was manifested by the change in viscosity readings as the stress was increased from 0.6 to 6.0 r.p.m. and then reversed from 6.0 to 0.6 r.p.m.

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Preformulation Studies II: Stability of Drug Substances in Solid Pharmaceutical Systems

J. TINGSTAD▲ and J. DUDZINSKI

Abstract □ The stability of drug substances in solid pharmaceutical systems is discussed. Theoretical models for various situations are proposed and their practical implications are considered.

Keyphrases □ Preformulation theory—stability of drug substances in solid pharmaceutical systems □ Drug stability in solid pharmaceutical systems—theory, preformulation □ Solid drug systems—stability of drug substance, theory

The stability of drug substances in solid pharmaceutical systems has been discussed in some detail previously (1–11 and the references cited therein). However, further treatment of the subject seems desirable for the following reasons.

1. In spite of the importance of solid dosage forms, there are relatively few quantitative reports on their chemical stability, primarily because of the complexities and difficulties involved.

2. This paper presents a somewhat different point of view than those of most references cited previously.

3. Some theoretical concepts need further clarification and application to practical stability studies.

DISCUSSION

Quantitative chemical stability studies on drug substances in solid dosage forms are difficult to perform for two primary reasons. First, analytical results tend to have more scatter because tablets and capsules are distinct dosage units rather than the true aliquots encountered with stability studies on drug substances in solution. Second, tablets and capsules are heterogeneous and discontinuous systems involving gas (air and water vapor), liquid (*e.g.*, adsorbed water), and solid phases, all of which can vary in concentration during an experiment.

Analytical error can be minimized by ensuring that content uniformity is satisfactory before initiating stability studies. The problems arising from the heterogeneity associated with these systems are more difficult to overcome, but the primary prerequisites for dealing with them are an awareness of their existence, an under-

standing of their nature, and an appreciation of their practical significance. Some of these problems are as follows.

1. There can be a considerable time lag before physical pseudo-equilibrium is established. For example, appreciable time may elapse before the concentration of water (liquid and vapor phases) comes to equilibrium (1). This is especially true for gelatin capsules, because water present in the capsule shell must equilibrate with that in the formulation and in the surrounding air space.

2. This equilibrium can be upset by opening the container to remove samples for assay, since water vapor can then escape from containers stored at elevated temperatures.

3. If the container is pervious to gaseous diffusion, the stability picture may become confused. For example, Table I shows that at 60°, a water-labile component of a tablet formulation was considerably more stable in a water-permeable blister package than in a sealed glass bottle; at room temperature and 70% relative humidity, the situation was reversed. The tablets, manufactured and packaged at room temperature, probably lose considerable water through the film at 60°, thereby improving stability. At room temperature and 70% relative humidity, the diffusion of water vapor is in the opposite direction.

4. A number of phase changes could take place during the stability study. For example, salicylic acid from aspirin hydrolysis would be in solution until the saturation point is reached and then a solid phase would separate. In addition, sublimation and subsequent deposition of salicylic acid crystals on the dosage form and surface of the container often take place.

5. Solid-state reactions (*e.g.*, oxidation by atmospheric oxygen) may occur simultaneously with solution reactions, and the degradation rates may not be additive (5).

6. Because of the low water volumes involved, kinetic studies may be complicated by chemical equilibrium phenomena and other factors (7).

7. An increase in temperature increases reaction rates and (usually) solubility; this would have a double effect on the overall degradation rate (12). However, this may be partially offset by the lowered concentration of liquid water at higher temperatures.

These problems can be minimized by observing four fundamental rules when conducting quantitative kinetic studies on solid dosage forms. First, as mentioned earlier, content uniformity must be within satisfactory limits. Second, tightly sealed glass containers should be used unless an alternative package is involved. Third, the amount of water present in the dosage form should be determined, since the rate of degradation often depends on the amount of water present. Furthermore, the equilibrium between liquid and vapor phases varies with temperature; consequently, it would be more accurate to determine water concentration at each storage temperature. One approach is to add a measured amount of water to the formulation (5), but part of this water vaporizes into the air space in the container. Consequently, water determinations done on the stored samples have more meaning (10), especially when elevated temperatures are involved.

The fourth rule is that a separate, sealed sample should be taken for each assay point and water determination. If not, water equilibrium can be upset when the container is opened to remove samples. If these rules are observed, meaningful data can be gathered in many cases. The treatment of some of these data may involve relatively complex mathematics (1, 7, 10), but for many systems a few basic, relatively simple, theoretical concepts will suffice. These concepts are as follows.

1. The three most common reactions encountered in stability studies on solid systems are: (*a*) hydrolysis in solution (*e.g.*, aspirin); (*b*) oxidation, either in solution or in the solid state (*e.g.*, vitamin A); and (*c*) reactions between two ingredients.

2. When both Class (*a*) and Class (*b*) reactions are involved, the reaction rates can be additive or antagonistic. For example (assuming no oxidation in solution), adding water to a dry system would initiate hydrolysis but could decrease solid-state oxidation, probably by water adsorption on solid surfaces (5).

3. Class (*a*) reactions are usually zero order in solid dosage forms unless all of the reactant is in solution; then first-order kinetics usually prevail. However, in some situations (*e.g.*, if an acidic or basic degradation product catalyzes the reaction), the kinetics are more complicated. Class (*b*) reactions can be of various orders, although they are often first order if sufficient oxygen is present. Class (*c*) reactions can be of various orders, depending on the concentrations and solubilities of the reactants.

Table I—Degradation of Water-Labile Component of a Tablet Formulation in Two Different Packages under Different Storage Conditions

Package	—Amount Lost (%) in 8 Weeks at— 60°	Room Temperature (70% Relative Humidity)
Blister	11.6	7.0
Glass	84.4	0

4. A particular model or system should be carefully defined. For example, the Class (*a*) reactions described here assume that the reactant dissolves in an adsorbed film of water and that the concentration of adsorbed water is directly proportional to the total amount of water present. The discussion here of Class (*b*) reactions is limited to oxidation in the solid state. Finally, the mathematics describing a Class (*c*) reaction would be different, depending on whether one assumed solid state or solution reactions.

5. Although the models described here are consistent with many reported data, this consistency does not prove that degradations are occurring *via* mechanisms proposed in the model. The mechanisms of degradation for each individual situation would have to be proven by the usual rigorous procedures.

Because of the relatively small amounts of water present in these systems, concentrations (in solution) of degradation products may become significant as the reaction proceeds; then chemical equilibrium phenomena may complicate the kinetics (7). Furthermore, in some cases the drug substance may be less stable in a solid system with a small amount of water present than when the same ingredients are dissolved or suspended in a relatively large quantity of water (10). Nevertheless, many solid systems behave predictably according to the first three concepts above, especially during the first 10–40% of the reaction. Therefore, it would be useful to take a more detailed look at those predictable situations. Only Class (*a*) and Class (*b*) reactions will be considered here.

Figure 1 illustrates the effect of adding water to a solid system involving a relatively insoluble drug substance that degrades only in aqueous solution [Class (*a*), with assumptions mentioned in the fourth concept above]. With no water present, no degradation occurs. As successive amounts of water are added (assuming all of it is available as solvent), the amount of drug substance degrading per unit time increases proportionally:

$$d = k_0 \times v \qquad \text{(Eq. 1)}$$

where, for example, d is the amount degraded in 1 day, k_0 is the apparent zero-order rate constant[1] (in milligrams per milliliter per day), and v is the volume of water (in milliliters) present in the solid system. For example, if $k_0 = 1$ mg./ml./day and $v = 0.1$ ml., then $d = 0.1$ mg.; then if the water content is doubled to 0.2 ml., d would also double. Since the point is never reached where all of the drug substance is in solution, the plot consists of a single straight line with a slope of k_0.

In Fig. 2, the same assumptions are made except that the drug is relatively soluble. Up to point A, the amount of water present is not enough to dissolve all of the drug substance and the situation is identical to that in Fig. 1. After point A, all of the drug substance is in solution and first-order kinetics prevail:

$$d = (1 - e^{-k_1}) \times c \times v \qquad \text{(Eq. 2)}$$

where d and v have the same meaning as in Eq. 1, c is the initial concentration of drug substance (in milligrams per milliliter), and k_1 is the first-order rate constant in reciprocal days. Here, adding more water does not change the value of d (ignoring other dilution effects) because, for example, doubling v automatically decreases c by one-half. Thus, the slope is (ideally) zero when first-order kinetics pre-

[1] As pointed out previously (12), the apparent zero-order rate constant k_0 is equal to the first-order rate constant k_1 times the solubility of the drug substance in the solvent at the experimental temperature. Consequently, when the temperature is raised in accelerated studies, k_0 is increased by two factors: the temperature effect on k_1 and the temperature effect on the solubility of the drug substance.

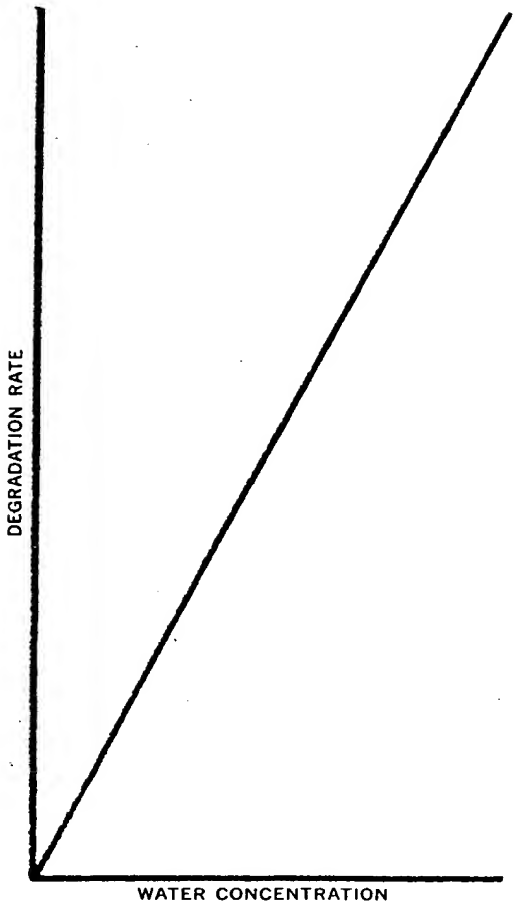

Figure 1—*Model plot showing the effect of water concentration on the stability of a relatively insoluble drug substance that degrades only in aqueous solution.*

vail and equal to k_0 when zero-order kinetics dominate. This concept was touched on by others (5, 11) and will be discussed later.

In Fig. 3, the drug substance is relatively insoluble and subject to both oxidation in the solid phase and hydrolysis in solution. At point A, with no water present, the effect of oxidation is evident; there is appreciable degradation in the absence of water. From A to B, hydrolysis enters the picture, but its effects are partially hidden because the two reactions are antagonistic. That is, the presence of water reduces the effects of oxidation (*e.g.*, by adsorbing on and

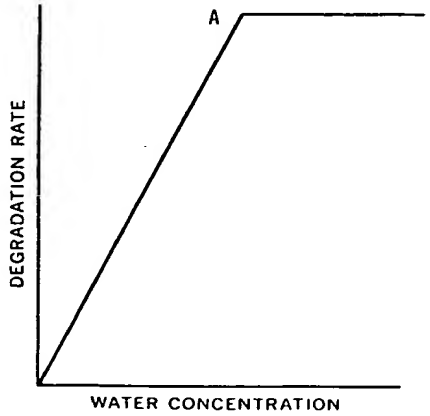

Figure 2—*Model plot showing the effect of water concentration on the stability of a relatively soluble drug substance that degrades only in aqueous solution. At point A, all of the drug substance is in solution.*

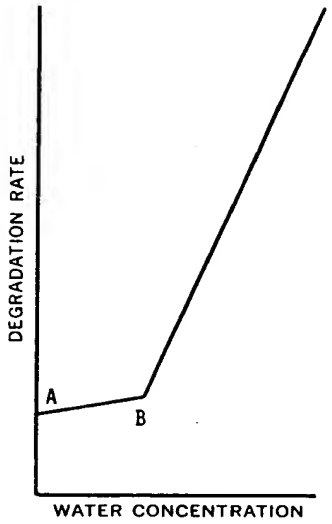

Figure 3—*Model plot showing the effect of water concentration on the stability of a relatively insoluble drug substance that degrades both in the solid state and in aqueous solution. See text for explanation.*

partially protecting the surface of the solid). After point B, the effects of oxidation become relatively constant and the situation more closely resembles that in Fig. 1. If the drug substance in Fig. 3 was water soluble, the plot would eventually plateau as in Fig. 2. Because a nonhydrolytic reaction is partially involved, the slope of the second portion of the plot is less likely to equal k_0. If the two reactions were simply additive, or if the hydrolytic reaction was much faster than the oxidative, the AB portion of the plot would be insignificant. The effect of hydrolysis alone could be determined by eliminating oxygen from the system.

In some situations, the data will not fit the models, but this is not unusual for theoretical models representing complex systems. The report on thiamine hydrochloride (10) is a case in point, but even here a good portion of the data fits the theoretical model. Examples from the literature and these laboratories can be used to illustrate the utility of the three models presented in Figs. 1–3.

Maulding *et al.* (11) obtained a plot identical to that in Fig. 1 using aspirin, a compound that fits the requirements of: (*a*) relatively low solubility, and (*b*) degradation primarily by hydrolysis. The zero-order nature of aspirin degradation in these systems is illustrated in that report, as well as in Fig. 4, based on data from these laboratories. In addition, the data of Leeson and Mattocks (1) can be plotted zero order, assuming an initial lag time (Fig. 5). Figure 6,

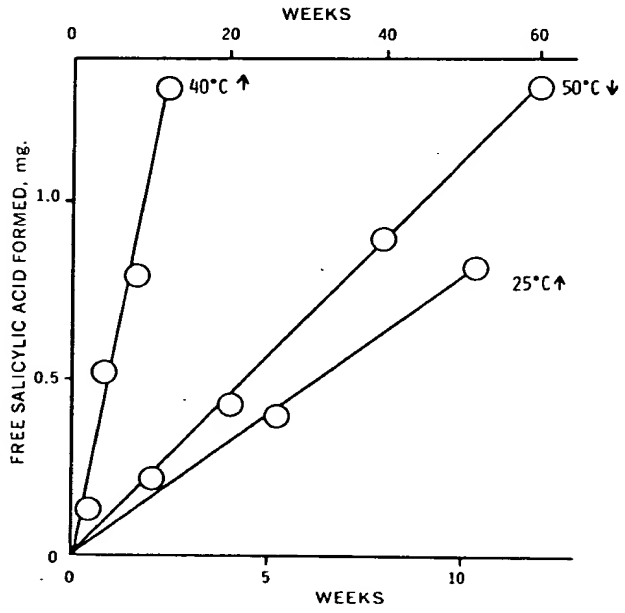

Figure 4—*Plot showing the zero-order nature of aspirin degradation in a solid system. The arrows show to which abscissa the plots refer.*

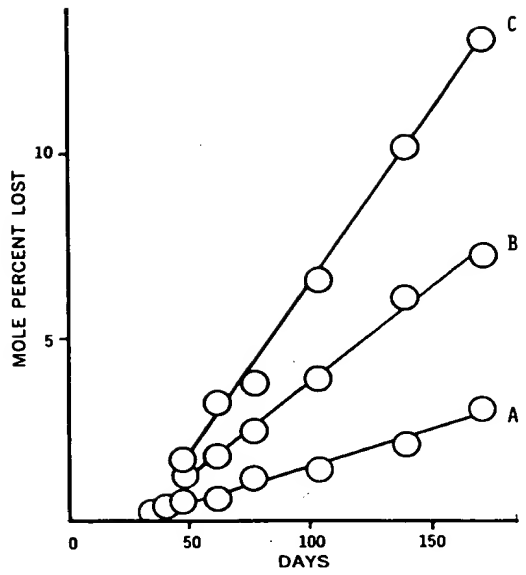

Figure 5—*Plot showing the zero-order nature of aspirin degradation in a solid system [50° data of Leeson and Mattocks (1)]. Key: A, 46.02 mm. vapor pressure of water; B, 68.30 mm.; and C, 74.20 mm.*

representing data from an experimental drug substance in these laboratories, also closely resembles Fig. 1.

The degradation of vitamin A in tablets was found to be first order rather than zero (2) but, as noted earlier, degradation involves more than simple hydrolysis. While the data of Tardif (3) were plotted first order, some of the plots (*e.g.*, thiamine and ascorbic acid at 50°) appear to exhibit zero-order characteristics. Furthermore, if all the thiamine was in solution (possible, especially at the higher temperatures), this would give first-order kinetics. These examples illustrate that many situations do fit the theory when examined in this way.

Thiamine hydrochloride meets the general criteria for compounds yielding the plot seen in Fig. 2. However, the situation is complicated by equilibrium phenomena and other factors, as discussed by Carstensen *et al.* (10). Nevertheless, a significant portion of the reaction follows zero-order kinetics (Fig. 7), and the plot in Fig. 4 of their report suggests that, under ideal conditions (*i.e.*, assuming no chemical equilibrium effects), a plot similar to that in Fig. 2 of this paper would result.

The situation represented in Fig. 3 finds a close parallel in the report of Carstensen *et al.* (5) on vitamin A acetate. Figure 8 is a plot of their data, and it is practically identical to the ideal case depicted in Fig. 3. The ordinate in Fig. 8 is the sum total of the vitamin A lost at all temperatures at a given water concentration. Data from 55°, 85° (4 days only), and 70° (8 days only) were not used because: (*a*) in some cases the concentration of vitamin A reached zero, and it is not known at which point in that time interval the value became zero; and (*b*) the second data points at 70 and 85° showed a drastic reduction in degradation rate, which raises questions about their validity (these data were also rejected by the authors in their calculations).

From Fig. 8 it is quite evident that in their system vitamin A

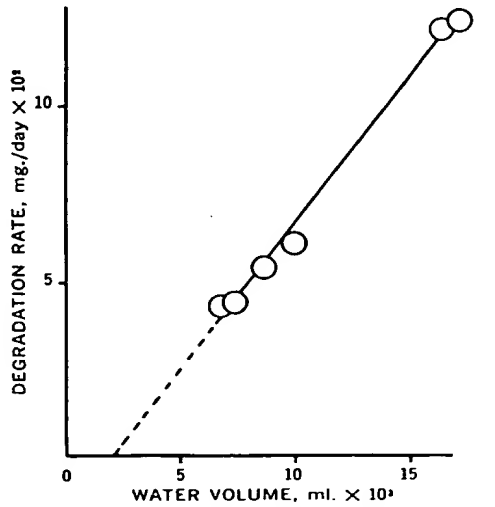

Figure 6—*Plot of actual data for experimental drug substance (confirms the validity of the model in Fig. 1).*

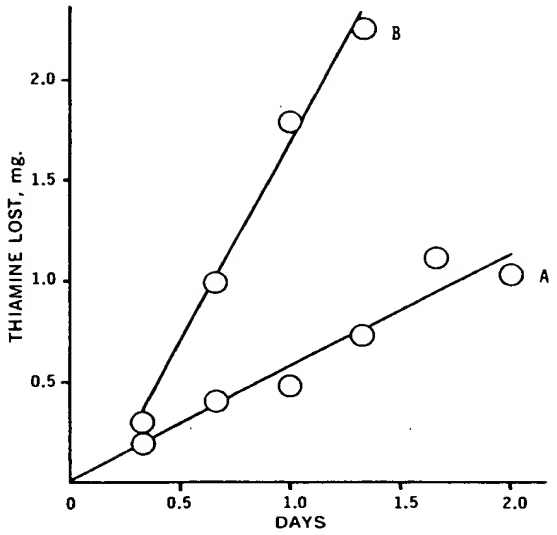

Figure 7—*Plot showing the zero-order nature of thiamine hydrochloride degradation in a solid system [55° data of Carstensen et al. (10)]. Key: A, 4% moisture; and B, 5% moisture.*

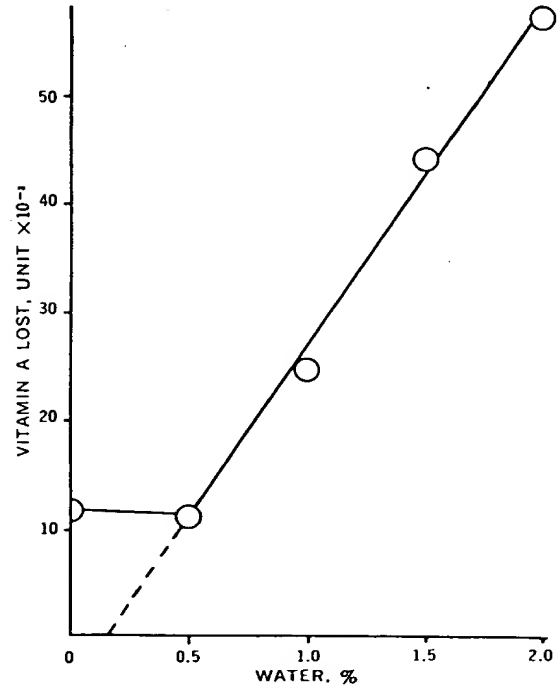

Figure 8—*Plot showing the effect of water concentration on the stability of vitamin A acetate in a solid system [data of Carstensen et al. (5)]. See text for explanation.*

acetate degrades significantly in the absence of water. When 0.5% water is added, the stability picture remains essentially the same, indicating that the water's contribution to degradation is offset by the partial protection it affords against solid-state oxidation. As more water is added, however, the degradation rate increases linearly with water concentration, indicating that the contribution of solid-state oxidation has plateaued and the effects of hydrolysis are becoming more evident.

The vitamin A acetate report (5) provides another example of the usefulness of the concepts and models presented in this paper. In discussing the theory behind their presentation, the authors (5) stated: "If vitamin A degrades by functional dependence of water content, then a $(1 + a)$ order reaction may be expressed as $dC_a/dt = -K \cdot C_A \cdot C_{H_2O}^a$ [their Eq. 1] where C denotes concentration and the subscripts denote vitamin A acetate and water." From their data, they reached the conclusion that the apparent value of a is 2–3, a relatively high order.

If one assumes that zero-order kinetics prevail for the water-dependent degradation (a reasonable assumption, since vitamin A acetate is relatively water insoluble), then their Eq. 1 reduces to our Eq. 1 when the value of a is unity. A slightly different interpretation of their data yields values of a that are close to unity and confirm the validity of the model in Fig. 3. That interpretation is as follows.

From the sharp break in the slope in Fig. 8, it is evident that the 0–0.5% range of water concentration is atypical. That is, their Eq. 1 assumes a continuous function and, for their system, the function can be considered continuous only between 0.5 and 2.0% added water. Therefore, the "water added" concept should, in our opinion, start from their 0.5% mark. Thus, their 0.5, 1.0, 1.5, and 2.0 values for "percent water added" should (more correctly) be taken as 0, 0.5, 1.0, and 1.5%, respectively. For example, their 1.5% is, more correctly, 1.0% (*i.e.*, 1.5 − 0.5). Recalculating their data using the corrected values of water added yields the following values for a: 85°, 0.90; 70°, 0.87; 55°, 1.45; 25°, 1.46; and 5°, 1.42. Considering the complexity of the system, experimental values of 0.87–1.46 (average = 1.2) against a theoretical value of unity is good confirmation of the validity of the model in Fig. 3.

CONCLUSION

Stability data for solid dosage forms, gathered under suitably controlled conditions, can often be successfully interpreted using the schematic models presented in Figs. 1–3.

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Sodium-Ion Electrode: Continuous Monitoring of Tablet Dissolution *via* Flowing Streams

T. E. NEEDHAM, Jr., L. A. LUZZI▲, and W. D. MASON

Abstract □ The use of a continuous flowing stream apparatus to follow tablet dissolution was studied. A dissolution chamber using a commercially available filter unit was designed to follow tablet dissolution through use of either a sodium-ion electrode or a spectrophotometric analytical module. The effect of variation of flow rate on the dissolution profile and the ability of the apparatus to differentiate between the common tablet parameters of hardness and drug potency were shown.

Keyphrases □ Tablet dissolution—determination in continuous-flow equipment using sodium-ion electrode or UV spectrophotometry differentiation between parameters of hardness and drug potency □ Dissolution equipment, tablets—continuous-flow system using sodium-ion electrode or UV spectrophotometry, differentiation between parameters of hardness and drug potency □ Sodium-ion electrode system—used to determine tablet dissolution in continuous-flow system

The importance of *in vitro* testing of tablet and capsule dissolution and its relationship to drug availability are accepted facts. The correlation of *in vitro* to *in vivo* data shows the need for a versatile and flexible apparatus which, through adjustment of its parameters to the variance in dissolution profiles of different tablets, can succeed in matching such data. A second but equally important consideration is a reduction in the time and labor required for such *in vitro* analysis. By using the technique of continuous analysis in flowing streams to monitor dissolution, the entire profile can be recorded and experimental error can be reduced to a minimum.

In recent years, equipment for studying the dissolution of solid dosage forms under conditions of continuous flow has been presented. The arguments for using a continuous-flow method and the theoretical considerations involved have been documented (1, 2). These authors suggested that the continuous-flow

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REVIEW ARTICLE

Pharmaceutical Salts

STEPHEN M. BERGE *‡, LYLE D. BIGHLEY *, and DONALD C. MONKHOUSE ˣ

Keyphrases □ Pharmaceutical salts—general pharmacy, physicochemical properties, bioavailability, pharmaceutical properties, toxicology, review □ Salts, pharmaceutical—general pharmacy, physicochemical properties, bioavailability, pharmaceutical properties, toxicology, review □ Physicochemical properties—dissolution, solubility, stability, and organoleptic properties of pharmaceutical salts, review □ Bioavailability—formulation effects, absorption alteration and pharmacokinetics of pharmaceutical salts, review □ Toxicology—pharmaceutical salts, review

CONTENTS

The chemical, biological, physical, and economic characteristics of medicinal agents can be manipulated and, hence, often optimized by conversion to a salt form. Choosing the appropriate salt, however, can be a very difficult task, since each salt imparts unique properties to the parent compound.

Salt-forming agents are often chosen empirically. Of the many salts synthesized, the preferred form is selected by pharmaceutical chemists primarily on a practical basis: cost of raw materials, ease of crystallization, and percent yield. Other basic considerations include stability, hygroscopicity, and flowability of the resulting bulk drug. Unfortunately, there is no reliable way of predicting the influence of a particular salt species on the behavior of the parent compound. Furthermore, even after many salts of the same basic agent have been prepared, no efficient screening techniques exist to facilitate selection of the salt most likely to exhibit the desired pharmacokinetic, solubility, and formulation profiles.

Some decision-making models have, however, been developed to help predict salt performance. For example, Walkling and Appino (1) described two techniques, "decision analysis" and "potential problem analysis," and applied them to the selection of the most suitable derivative of an organic acid for development as a tablet. The derivatives considered were the free acid and the potassium, sodium, and calcium salts. Both techniques are based on the chemical, physical, and biological properties of these specific derivatives and offer a promising avenue for developing optimal salt forms.

Information on salts is widely dispersed throughout the pharmaceutical literature, much of which addresses the use of salt formation to prolong the release of the active component, thereby eliminating various undesirable drug properties (2–6). This review surveys literature of the last 25 years, emphasizing comparisons between the properties of different salt forms of the same compound. Included also is a discussion of potentially useful salt forms. Our purpose is twofold: to present an overview of the many different salts from which new drug candidates can be chosen and

Table I—FDA-Approved Commercially Marketed Salts

Anion	Percent[a]	Anion	Percent[a]
Acetate	1.26	Iodide	2.02
Benzenesulfonate	0.25	Isethionate[i]	0.88
Benzoate	0.51	Lactate	0.76
Bicarbonate	0.13	Lactobionate	0.13
Bitartrate	0.63	Malate	0.13
Bromide	4.68	Maleate	3.03
Calcium edetate	0.25	Mandelate	0.38
Camsylate[b]	0.25	Mesylate	2.02
Carbonate	0.38	Methylbromide	0.76
Chloride	4.17	Methylnitrate	0.38
Citrate	3.03	Methylsulfate	0.88
Dihydrochloride	0.51	Mucate	0.13
Edetate	0.25	Napsylate	0.25
Edisylate[c]	0.38	Nitrate	0.64
Estolate[d]	0.13	Pamoate (Embonate)	1.01
Esylate[e]	0.13	Pantothenate	0.25
Fumarate	0.25	Phosphate/diphosphate	3.16
Gluceptate[f]	0.18	Polygalacturonate	0.13
Gluconate	0.51	Salicylate	0.88
Glutamate	0.25	Stearate	0.25
Glycollylarsanilate[g]	0.13	Subacetate	0.38
Hexylresorcinate	0.13	Succinate	0.38
Hydrabamine[h]	0.25	Sulfate	7.46
Hydrobromide	1.90	Tannate	0.88
Hydrochloride	42.98	Tartrate	3.54
Hydroxynaphthoate	0.25	Teoclate[j]	0.13
		Triethiodide	0.13

Cation	Percent[a]	Cation	Percent[a]
Organic:		Metallic:	
Benzathine[k]	0.66	Aluminum	0.66
Chloroprocaine	0.33	Calcium	10.49
Choline	0.33	Lithium	1.64
Diethanolamine	0.98	Magnesium	1.31
Ethylenediamine	0.66	Potassium	10.82
Meglumine[l]	2.29	Sodium	61.97
Procaine	0.66	Zinc	2.95

[a] Percent is based on total number of anionic or cationic salts in use through 1974. [b] Camphorsulfonate. [c] 1,2-Ethanedisulfonate. [d] Lauryl sulfate. [e] Ethanesulfonate. [f] Glucoheptonate. [g] *p*-Glycollamidophenylarsonate. [h] *N,N'*-Di(dehydroabietyl)ethylenediamine. [i] 2-Hydroxyethanesulfonate. [j] 8-Chlorotheophyllinate. [k] *N,N'*-Dibenzylethylenediamine. [l] *N*-Methylglucamine.

to assemble data that will provide, for the student and practitioner alike, a rational basis for selecting a suitable salt form.

POTENTIALLY USEFUL SALTS

Salt formation is an acid–base reaction involving either a proton-transfer or neutralization reaction and is therefore controlled by factors influencing such reactions. Theoretically, every compound that exhibits acid or base characteristics can participate in salt formation. Particularly important is the relative strength of the acid or base—the acidity and basicity constants of the chemical species involved. These factors determine whether or not formation occurs and are a measure of the stability of the resulting salt.

The number of salt forms available to a chemist is large; surveys of patent literature show numerous new salts being synthesized annually. Various salts of the same compound often behave quite differently because of the physical, chemical, and thermodynamic properties they impart to the parent compound. For example, a salt's hydrophobicity and high crystal lattice energy can affect dissolution rate and, hence, bioavailability. Ideally, it would be desirable if one could predict how a pharmaceutical agent's properties would be affected by salt formation.

Tables I and II list all salts that were commercially marketed through 1974. The list was compiled from all agents listed in "Martindale The Extra Pharmacopoeia," 26th ed. (7). Table I categorizes all salt forms approved by the Food and Drug Administration (FDA), while Table II lists those not approved by the FDA but in use in other countries. (Only salts of organic compounds are considered because most drugs are organic substances.) The relative frequency with which each salt type has been used is calculated as a percentage, based on the total number of anionic or cationic salts in use through 1974. Because of simple availability and physiological reasons, the monoprotic hydrochlorides have been by far the most frequent choice of the available anionic salt-forming radicals, outnumbering the sulfates nearly six to one. For similar reasons, sodium has been the most predominant cation.

Knowledge that one salt form imparts greater water solubility, is less toxic, or slows dissolution rate would greatly benefit chemists and formulators. In some cases, such generalizations can be made. Miller and Heller (8) discussed some properties associated with specific classes of salt forms. They stated that, in general, salt combinations with monocarboxylic acids are insoluble in water and lend themselves to repository preparations, while those of dicarboxylic acids confer water solubility if one carboxylic group is left free. Pamoic acid, an aromatic dicarboxylic acid, is an exception since it is used as a means of obtaining prolonged action by forming slightly soluble salts with certain basic drugs. Saias *et al.* (9) reviewed the use of this salt form in preparing sustained-release preparations. More recently, latentiation of dihydrostreptomycin (10)

Table II—Non-FDA-Approved Commercially Marketed Salts

Anion	Percent[a]
Adipate	0.13
Alginate	0.13
Aminosalicylate	0.25
Anhydromethylenecitrate	0.13
Arecoline	0.13
Aspartate	0.25
Bisulfate	0.25
Butylbromide	0.13
Camphorate	0.13
Digluconate	0.13
Dihydrobromide	0.13
Disuccinate	0.13
Glycerophosphate	0.88
Hemisulfate	0.13
Hydrofluoride	0.13
Hydroiodide	0.25
Methylenebis(salicylate)	0.13
Napadisylate[b]	0.13
Oxalate	0.25
Pectinate	0.13
Persulfate	0.13
Phenylethylbarbiturate	0.13
Picrate	0.13
Propionate	0.13
Thiocyanate	0.13
Tosylate	0.13
Undecanoate	0.13
Cation	**Percent[a]**
Organic:	
Benethamine[c]	0.33
Clemizole[d]	0.33
Diethylamine	0.33
Piperazine	0.98
Tromethamine[e]	0.33
Metallic:	
Barium	0.33
Bismuth	0.98

[a] Percent is based on total number of anionic and cationic salts in use through 1974. [b] 1,5-Naphthalenedisulfonate. [c] *N*-Benzylphenethylamine. [d] 1-*p*-Chlorobenzyl-2-pyrrolidin-1′-ylmethylbenzimidazole. [e] Tris(hydroxymethyl)aminomethane.

using pamoic acid resulted in the formation of a delayed-action preparation. Numerous studies using pamoate salts are dispersed throughout the literature (11–15).

Alginic acid also has been used to prepare long-acting pharmaceuticals. Streptomycin alginate was prepared (16) and shown to be effective in sustained-release preparations. A striking example of a long-acting alginate salt is that of pilocarpine. When dispersed in sterile water and dried to a solid gel, this compound was found useful in the preparation of long-acting ophthalmic dosage forms (17). While liquid preparations of the alginate and hydrochloride salts possess similar miotic activity, studies showed that solid pilocarpine alginate flakes constricted pupil size more effectively and increased the duration of miosis significantly when compared with the liquid preparations. Solid dose pilocarpine may be more uniformly available, because it diffuses more slowly through the gel matrix which holds the drug in reserve. In contrast, drops of the commonly employed solution dosage form release the dose immediately to the conjunctival fluid.

Málek *et al.* (18) devised a unique way of prolonging action through salt formation; they showed that the distribution of several antibiotics could be markedly altered by merely preparing macromolecular salts. Since macromolecules and colloidal particles have an affinity for the lymphatic system, streptomycin, neomycin, viomycin, and streptothrycin were combined with high molecular weight compounds such as polyacrylic acids, sulfonic or phosphorylated polysaccharides, and polyuronic derivatives. Parenteral administration of these compounds produced low blood levels of the antibiotic for long periods, while lymph levels were high. (In comparison, streptomycin sulfate gave high blood levels but low lymph levels.) This alteration in distribution caused the streptomycin to prolong its passage through the body, since lymphatic circulation is quite slow.

The appropriate choice of a salt form has been found to reduce toxicity. It can be rationalized that any compound associated with the normal metabolism of food and drink must be essentially nontoxic. The approach of choosing organic radicals that are readily excreted or metabolized opened up a new class of substances from which to select a salt form. For example, certain salts of the strong base choline have proven to be considerably less toxic than their parent compound. The preparation and properties of choline salts of a series of theophylline derivatives were reported (19), and it was shown that choline theophyllinate possessed a greater LD_{50} than theophylline or its other salts (20). It was postulated that this agent would be less irritating to the GI tract than aminophylline, because "its basic constituent, choline, is an almost completely nontoxic substance of actual importance to the physiologic economy." This evidence led to the preparation of choline salicylate (21) as an attempt to reduce the GI disturbances associated with salicylate administration. Clinical studies indicated that choline salicylate elicited a lower incidence of GI distress, was tolerated in higher doses, and was of greater benefit to the patient than was acetylsalicylic acid (aspirin).

Amino acids and acid vitamins also have been used as salt-forming agents. Based on the evidence that coadministration of amino acids with aminoglycoside antibiotics reduced their toxicity, a series of amino acid salts of dihydrostreptomycin was prepared (22). In all but one case, the acute toxicities of these salts were lower than the toxicity of the sulfate. The ascorbate and pantothenate also were synthesized and shown to be less toxic than the sulfate. Of the salts prepared, the ascorbate had the highest LD_{50}.

The vitamins most commonly used for forming salts exhibiting reduced toxicity are ascorbic and pantothenic acids. Keller *et al.* (23) were the first to use pantothenic acid as a means of "detoxifying" the basic streptomyces antibiotics. Parenteral administration of the pantothenates of streptomycin and dihydrostreptomycin had a significantly reduced incidence of acute neurotoxicity in cats as compared with the sulfates. Subsequent studies (24–28) supported this finding and showed that the pantothenates of neomycin and viomycin also are less toxic. The ascorbate of oleandomycin was synthesized and its pharmacological properties were reported (29). Upon intramuscular injection in rats, it produced less irritation than the phosphate.

p-Acetamidobenzoic acid, an innocuous metabolite of folic acid present in normal blood and urine, has been used in preparing salts. In particular, it yields stable salts with amines that otherwise tend to form hygroscopic products with conventional acid components (30).

Often the salt form is chosen by determining a salt

component that will pharmacologically antagonize an unfavorable property or properties exhibited by the basic agent. Salts of *N*-cyclohexylsulfamic acid are an example of the practical application of this approach. *N*-Cyclohexylsulfamic acid salts, better known as cyclamates, have a characteristic sweet, pleasing taste. Although presently under investigation by the FDA for potentially carcinogenic properties, salts incorporating this compound can render unpleasant or bitter-tasting drugs acceptable. For example, the cyclamates of dextromethorphan and chlorpheniramine exhibit greatly improved bitterness thresholds compared to commonly occurring salts (31). Furthermore, their stability in aqueous solution was described as good when maintained at a pH not greater than 4.

N-Cyclohexylsulfamic acid salts of thiamine hydrochloride and lincomycin also have been synthesized. Thiamine *N*-cyclohexylsulfamate hydrochloride was reported to have a more pleasant taste than other thiamine salts while having an equal or greater stability (32). Lincomycin cyclamate, shown to possess an enhanced thermal stability over its hydrochloride, was prepared (33) to test the hypothesis that reduced lincomycin absorption in the presence of small quantities of cyclamates was due to a simple metathetic reaction. However, this assumption was found not to be true. An extensive study of the preparation and characterization of cyclamic acid salts of several widely used classes of drugs including antihistamines, antibiotics, antitussives, myospasmolytics, and local anesthetics was reported (34, 35).

Various salts of penicillin and basic amine compounds have been formulated in an effort to produce a long-acting, nonallergenic form of penicillin. Since antihistamines appear to mitigate the symptomatology of penicillin reactions in some patients, coadministration of the two has been advocated. The preparation of the benzhydralamine salt of penicillin was an attempt to produce a repository form of penicillin with antiallergic properties (36). Blood levels achieved with this salt were comparable to those of penicillin G potassium; however, its antiallergic properties were not evaluated. In fact, the investigators noted that antihistamines can actually cause sensitization at times and stated that "despite their occasionally favorable influence on the symptoms of penicillin sensitivity, they contribute directly to the potential of drug sensitivity when co-administered with penicillin."

Silver salts of sulfanilamide, penicillin, and other antibiotics have been prepared and represent cases where the species (ions) are complementary. When aqueous solutions of the salts were applied topically to burned tissue, they yielded the combined benefits of the oligodynamic action of silver and the advantages of the antibacterial agents (37).

The use of 8-substituted xanthines, particularly the 8-substituted theophyllines, as salt-forming agents was first reported in the preparation of a series of antihistamine salts (38–41). Synthesis of these xanthine salts was an attempt to find a drug to counteract the drowsiness caused by the antihistamines with the stimulant properties of the xanthines. When an electronegative group is introduced into the xanthine molecule at the 8-position, the electron-drawing capacity of the substituent results in the creation of an acidic hydrogen at position 7. Thus, these moderately strong acidic compounds can undergo salt formation with various organic bases.

The 8-halotheophyllines were the first group of xanthines studied as potential salt-forming agents. Since the report on the preparation of the 8-chlorotheophylline salt of diphenhydramine (42), synthesis of the 8-halotheophyllinates of a number of organic bases has been attempted. The 8-chlorotheophylline salts of quinine, ephedrine, and strychnine were prepared and characterized (43). These salts were less water soluble than the corresponding free alkaloidal bases. In a similar report, the 8-chlorotheophyllinates of three synthetic narcotics, meperidine, levorphanol, and metopon, were prepared (44).

Pharmacological and clinical studies involving the 8-bromotheophylline pyrilamine salt revealed the unusual diuretic properties associated with the 8-halotheophylline portion of the compound (45, 46). This finding initiated an investigation into the preparation of a soluble 8-bromotheophylline salt of high diuretic activity. With readily available amines, over 30 salts were synthesized and screened for diuretic activity (47). When tested against theophylline salts of the same amines, the 8-bromotheophyllinates showed greater activity in every case.

With the successful formation of 8-halotheophyllinates of organic bases, Morozowich and Bope (48) proposed that, if the halogen moiety was replaced with a more electronegative substituent such as a nitro group, a more acidic compound would be formed. Presumably, more stable salts would result and precipitation of the free xanthine derivative in the stomach would be less likely to occur. On this premise, they successfully prepared pharmacologically effective 8-nitrotheophyllinates of several pharmaceutically useful bases.

Duesel *et al.* (19), in their study of choline theophyllinate, prepared the 8-chloro-, 8-bromo-, and 8-nitrotheophylline salts of choline. Oral toxicity studies in mice showed that the LD_{50} of the 8-nitrotheophyllinate was much greater than that of either 8-halotheophylline. In fact, it remained nonlethal at doses as high as 5 g.

Polygalacturonic acid, a derivative of pectin, has been used to prepare quinidine salts exhibiting reduced toxicity (49, 50). The compound possesses special demulcent properties and inhibits mucosal irritation. The rationale for use of this agent is to reduce the ionic shock to the GI mucosa resulting from the flood of irritating ions liberated by rapid dissociation of the conventional inorganic quinidine salts. Studies have shown that it is four times less toxic orally than the sulfate. This difference was attributed to the slower release of quinidine from the polygalacturonate.

Other compounds reported to be potentially useful as pharmaceutical salt forms are listed in Table III.

PHYSICOCHEMICAL STUDIES

Biological activity of a drug molecule is influenced by two factors: its chemical structure and effect at a specific site and its ability to reach—and then be removed from—the site of action. Thus, a knowledge of the physicochemical properties of a compound that influence its absorption, distribution, metabolism, and excretion is essential for a complete understanding of the onset and duration of ac-

Table III—Potentially Useful Salt Forms of Pharmaceutical Agents

Salt-Forming Agent	Compound Modified	Modification	Reference
Acetylaminoacetic acid	Doxycycline	Solubility	51
N-Acetyl-L-asparagine	Erythromycin	Solubility, activity, stability	52
N-Acetylcystine	Doxycycline	Combined effect useful in pneumonia	53
Adamantoic acid	Alkylbiguanides	Prolonged action	54
Adipic acid	Piperazine	Stability, toxicity, organoleptic properties	55
N-Alkylsulfamates	Ampicillin	Absorption (oral)	56
	Lincomycin	Solubility	57
Anthraquinone-1,5-disulfonic acid	Cephalexin	Stability, absorption	58
Arabogalactan sulfate (arabino)	Various alkaloids	Prolonged action	59, 60
Arginine	Cephalosporins	Toxicity	61
	α-Sulfobenzylpenicillin	Stability, hygroscopicity, toxicity	62
Aspartate	Erythromycin	Solubility	63
Betaine	Tetracycline	Gastric absorption	64
Bis(2-carboxychromon-5-yloxy)alkanes	7-(Aminoalkyl)theophyllines	Activity, prolonged prophylactic effect	65
Carnitine	Metformin	Toxicity	66
4-Chloro-*m*-toluenesulfonic acid	Propoxyphene	Organoleptic properties	67
Decanoate	Heptaminol	Prolonged action	68
Diacetyl sulfate	Thiamine	Stability, hygroscopicity	69
Dibenzylethylenediamine	Ampicillin	Prolonged action	70, 71
Diethylamine	Cephalosporins	Reduced pain on injection	72
Diguaiacyl phosphate	Tetracycline	Activity	73
Dioctyl sulfosuccinate	Vincamine	Organoleptic properties	74
Embonic (pamoic) acid	Kanamycin	Toxicity	75
	2-Phenyl-3-methylmorpholine	Toxicity	76
Fructose 1,6-diphosphoric acid	Tetracycline	Solubility	77
	Erythromycin	Solubility	
Glucose 1-phosphoric acid, glucose 6-phosphoric acid	Tetracycline	Solubility	77
	Erythromycin	Solubility, activity, stability	52
L-Glutamine	Erythromycin	Toxicity	78
Hydroxynaphthoate	Bephenium	Prolonged action	79
2-(4-Imidazolyl)ethylamine	Prostaglandin	Stability	80
Isobutanolamine	Theophylline	Organoleptic properties	81
Lauryl sulfate	Vincamine	Toxicity, stability, hygroscopicity	62
Lysine	α-Sulfobenzylpenicillin		61
	Cephalosporins	Solubility	82
Methanesulfonic acid	Pralidoxime (2-PAM)	Toxicity, stability, hygroscopicity	62
N-Methylglucamine	α-Sulfobenzylpenicillin	Reduced pain on injection	72
	Cephalosporins	Toxicity, faster onset of action	83
N-Methylpiperazine	Phenylbutazone	Reduced pain on injection	72
Morpholine	Cephalosporins	Organoleptic properties	84
2-Naphthalenesulfonic acid	Propoxyphene	Prolonged action	68
Octanoate	Heptaminol	Organoleptic properties	85
Probenecid	Pivampicillin	Prolonged action	86, 87
Tannic acid	Various amines	Activity	88
Theobromine acetic acid	Propoxyphene	Organoleptic properties	89
3,4,5-Trimethoxybenzoate	Tetracycline	Prolonged action	68
	Heptaminol	Absorption (oral)	90
Tromethamine	Aspirin	Physical state	91
	Dinoprost (prostaglandin $F_{2\alpha}$)		

tion, the relative toxicity, and the possible routes of administration (2).

In a review in 1960, Miller and Holland (92) stated that "different salts of the same drug rarely differ pharmacologically; the differences are usually based on the physical properties." In a subsequent review (93), Wagner expanded upon this statement, asserting that, although the nature of the biological responses elicited by a series of salts of the same parent compound may not differ appreciably, the intensities of response may differ markedly.

The salt form is known to influence a number of physicochemical properties of the parent compound including dissolution rate, solubility, stability, and hygroscopicity. These properties, in turn, affect the availability and formulation characteristics of the drug. Consequently, the pharmaceutical industry has systematically engaged in extensive preformulation studies of the physicochemical properties of each new drug entity to determine the most suitable form for drug formulation. Published information concerning such studies, however, is sparse. Preformulation studies have been outlined, and the influence of the salt form on the volatility and hygroscopicity of an agent under investigation was discussed (94).

In one such study, methylpyridinium-2-aldoxime (pralidoxime) salts were investigated (95). This study set out to prepare a salt with water solubility adequate to allow intramuscular injection of a low volume (2–3 ml) therapeutic dose. The original compound, the methiodide, had the disadvantages of limited aqueous solubility and high potential toxicity, since its high iodide content could result in iodism. On the basis of physiological compatibility, better water solubility, favorable stability, and relatively high percentage of oxime, the chloride salt of pralidoxime was selected for therapeutic administration; it was claimed that "the anion used to form the salt can confer physical properties of importance and significance for the formulation and administration of the compound" (95).

Some physicochemical properties of a series of mineral acid salts of lidocaine also were determined (96). While the hydrochloride and hydrobromide were more hygroscopic, they were more soluble in a number of solvents than the nitrate, perchlorate, phosphate, or sulfate salts.

Dissolution Rate—The dissolution rate of a pharmaceutical agent is of major importance to the formulator. In many cases, particularly with poorly soluble drugs, this characteristic best reflects the bioavailability of the com-

pound. As a rule, a pharmaceutical salt exhibits a higher dissolution rate than the corresponding conjugate acid or base at an equal pH, even though they may have the same equilibrium solubility. The explanation for this result lies in the processes that control dissolution.

Dissolution can be described by a diffusion layer model[1] in terms of an equation developed by Nernst and Brunner (97):

$$\frac{dW}{dt} = \frac{DS}{h}(C_s - C) \qquad \text{(Eq. 1)}$$

where W is the mass of the solute dissolved at time t, dW/dt is the rate of mass transfer per unit time, D is the solute molecule diffusion coefficient, S is the surface area of the dissolving solid, h is the diffusion layer thickness, C is the concentration of the drug in the bulk solution at time t, and C_s is the saturation solubility of the solute in the diffusion layer.

The driving force for dissolution in Eq. 1 is the difference between the saturation solubility of the drug and the concentration of the drug in the bulk fluid. If the drug is not rapidly absorbed after it dissolves, then C, the concentration in the bulk solution, approaches C_s and the dissolution rate is retarded. When this occurs, absorption is "absorption rate" limited (or "membrane transport" limited). If the absorption rate is rapid (or if the absorption mass transfer coefficient is much larger than DS/h of Eq. 1), however, C becomes negligible compared to C_s and dissolution occurs under "sink" conditions. Absorption is then said to be dissolution rate limited, which is what occurs with most poorly soluble drugs. In either case, an increase in C_s, as in salt formation, increases dissolution.

Salts often speed dissolution by effectively acting as their own buffers to alter the pH of the diffusion layer, thus increasing the solubility of the parent compound, C_s, in that layer over its inherent solubility at the pH of the dissolution medium. Hence, dissolution is controlled by solubility in the diffusion layer which, in turn, is determined by the pH of that layer. The influence of K_{sp} on the solubility term, C_s, and dissolution rate, should an accumulation of ions be allowed to occur, will be treated later.

Nelson (98), in a study of theophylline salts, was the first to show the correlation between diffusion layer pH and dissolution rate. The major impact that this study had on the pharmaceutical sciences was its conclusion that, if other factors remained constant, the dissolution rate of a compound determined the rate of buildup of blood levels with time and the maximum levels obtained. Those salts of the acidic theophylline with high diffusion layer pH's had greater *in vitro* dissolution rates than those exhibiting a lower diffusion layer pH. And, indeed, the rank order of dissolution rates correlated well with clinically determined blood levels. Presumably, the higher pH in the diffusion layer retards hydrolysis of the salt, thereby maintaining the anionic charge of the theophyllinate ion. This report led to many additional studies which illustrate the influence of the salt form on dissolution and the beneficial effects of changing nonionized drugs into salts.

Juncher and Raaschou (99) demonstrated that the rank order of peak blood levels of penicillin V, obtained upon administration of three different salts and the free acid, was the same as the rank order of their rates of dissolution *in vitro*. While the investigators ascribed these differences to the solubility properties of the salts, their experiments actually compared dissolution rates, not solubilities. The relative order of dissolution rates and mean maximal blood levels was: potassium salt > calcium salt > free acid > benzathine salt.

Nelson (100) determined dissolution rates for several weak acids and their sodium salts in media whose pH's represented GI fluids. In all cases, the sodium salt dissolved more rapidly than the free acid. This finding resolved the misconception that absorption of drugs is related only to solubility in the appropriate medium; rather, solubility affects absorption only to the extent that it affects dissolution rate. Absorption of drugs is a dynamic process, and the ultimate solubility of a drug in fluid at absorption sites is of limited consequence since absorption prevents the attainment of saturated solutions. Therefore, dissolution rate, more than solubility, influences absorption since it is a preceding process.

In two subsequent studies, Nelson and coworkers further illustrated the effects of changing nonionized drugs into salts. A report concerning tolbutamide (101), a weak acid, showed that the initial dissolution rate of tolbutamide sodium was approximately 5000 times more rapid than the free acid in acidic media and 300 times more rapid in neutral media. This difference, measured *in vitro*, reflected the differences observed between the free acid and the salt when administered to human subjects. Oral administration of tolbutamide sodium produced an immediate drop in blood sugar comparable to that produced by intravenous injection of the salt, while the slowly dissolving tolbutamide produced a smooth, sustained fall in blood sugar (102).

Correlation of urinary excretion rates and dissolution rates of tetracycline and some of its acid salts also was demonstrated by Nelson (103). The salts that exhibited greater *in vitro* dissolution rates showed greater urinary excretion rates, indicating more rapid absorption.

Benet (104), in a discussion of the biopharmaceutical basis for drug design, referred to the influence of the salt form on dissolution. He compared the dissolution rates of tetracycline and tolbutamide and their salts, as reported in the studies previously cited, and explained why the rates differ at the pH's exhibited by physiological fluids.

Although salt formation usually increases the dissolution rate of a drug, studies with aluminum acetylsalicylate (105, 106), warfarin sodium (107), and benzphetamine pamoate (108) showed that administration of the salt *slowed* dissolution of the drug and subsequent absorption compared to the nonionized form. This decrease appeared to result from precipitation of an insoluble particle or film on the surface of the tablet. Such a phenomenon decreases the effective surface area and prevents deaggregation of the particles. Theoretical considerations of the processes controlling dissolution of an acid salt of a base (108) and the sodium salt of a weak acid (109, 110) in reactive media have been discussed.

Tablet processing and various formulation factors can decrease the dissolution rate of a salt in human gastric juice over its nonionized form (111). Granulation and tableting caused the dissolution rate of phenobarbital sodium to

[1] The authors recognize the existence of other models; this one was chosen simply for illustrative purposes.

decrease but had the opposite effect on phenobarbital. Therefore, as a tablet dosage form, the dissolution rate of the sodium salt was slower than that of the free acid. These results were attributed to differences in the disintegrating properties of the tablets; in some instances, rapid dissolution may in fact be a problem for very soluble drugs.

Others have illustrated a phenomenon that decreases the dissolution rate of a salt below that of its nonionized form. Lin *et al.* (112) studied the relationship between salts and biological activity by dissolving three salts and the free base of an experimental antihypertensive in water, 0.1 *N* HCl, and pH 7.2 phosphate buffer. The dissolution rate of the monohydrochloride salt was lower than that of the free base in 0.1 *N* HCl and higher than the free base in both water and phosphate buffer. These authors ascribed this variation to the common ion effect and substantiated it experimentally. Although the biological activity of the monohydrochloride was greater than that of the free base, the implications of altered absorption characteristics on the activity of any other hydrochloride salt in GI fluids must be considered. Similar results also were reported for hydrochloride salts of some tetracyclines (113).

Some consideration must be given to the influence of salt formation on oral toxicity, which often reflects the relationship between the *in vivo* dissolution rate and the appearance of drug in the circulation (114, 115). Morozowich *et al.* (114) showed that the relative toxicities of a series of salts of a drug reflect the rate of absorption, providing the salt-forming agents themselves are relatively nontoxic. They stated that "when absorption is rate-limited by dissolution of the salt in the gastrointestinal tract, as will be the case with slowly soluble salts, the toxicity of a slowly dissolving salt will most probably be lower than that of a more rapidly dissolving salt." The implications of salt formation on toxicology will be discussed under *Toxicological Considerations*.

Several reviews dealt with the influence of the dissolution rate on drug availability and, in particular, salt effects (116, 117). Other reports illustrating the influence of salts and salt form on dissolution rate are listed in Table IV.

Solubility—Knowledge of the solubility characteristics of a pharmaceutical agent is essential, because solubility is usually an important factor in the pharmacokinetic profile, the chemical stability, and, ultimately, the formulation of the drug. As discussed previously, it is certainly a primary factor in controlling dissolution rates. The solubility of a compound depends basically upon the physical and chemical properties of the solute; *e.g.*, a lower melting point for a compound within a series reflects a decreased lattice energy, which would suggest a higher solubility. Solubility depends as well upon such elements as temperature, pressure, solvent properties (such as resulting pH), and, to a lesser extent, the state of subdivision of the solute.

An important solvent property which is often overlooked involves the common ion effect; in particular, hydrochloride salts of drugs often exhibit less than desirable solubility in gastric juice because of the abundance of chloride ions. The equilibrium involved is shown in Scheme I.

$$DH^+Cl^-_{solid} \overset{K_{sp}}{\rightleftharpoons} (DH^+)_{aq} + (Cl^-)_{aq}$$

Scheme I

Salt formation is perhaps one of the first approaches considered as a means of increasing a compound's water solubility. As with dissolution rates, however, salt formation does not always confer greater solubility. Liberally dispersed throughout the pharmaceutical literature are studies that compare the solubilities of different salt forms of the same compound with those of its free acid or base (Table IV). Selection of the salt form exhibiting the desired solubility properties is critical, since these properties often dictate the formulation characteristics of the drug.

Table IV—Additional References on Salt Form and Dissolution Rate

Topic	Reference
Dissolution rate of mixtures of weak acids and tribasic sodium phosphate	118
Physiological availability and *in vitro* dissolution characteristics of some solid dosage formulations of aminosalicylic acid and its salts	119
Biopharmaceutics, rate of dissolution: chronological bibliography	120
Biopharmaceutics: rate of dissolution *in vitro* and *in vivo*	121
Dissolution tests and interpretation of anomalies observed in the dissolution process of sulfaquinoxaline based on salt formation	122
Influence of the dissolution rate of lithium tablets on side effects	123
Dissolution kinetics of drugs in human gastric juice	124
Comparison of dissolution and absorption rates of different commercial aspirin tablets	125
In vitro dissolution rates of aminorex dosage forms and their correlation with *in vitro* availability	126

Phase solubility techniques were used to study the formation of complex salts of triamterene (127). The results indicated that the organic acid salts of basic drugs, such as amines, were more soluble in water than the corresponding inorganic (halide) salts. This consideration is important in the synthesis and selection of a salt form that will exhibit enhanced bioavailability and desirable formulation characteristics.

The hydrogen-ion concentration can significantly affect the solubility of a salt. Anderson (128) discussed the influence of pH on the solubility of pharmaceuticals. Mathematical relationships between pH and solubility of therapeutically useful weak acids and bases and their salts were given along with some considerations in the formulation of solutions of these particular agents.

An extensive study on the solubility interrelationships of the hydrochloride and free base of two pharmaceutically useful amines was reported (129). Mathematical equations describing the total solubility at an arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to experimental data with good results. This report elucidated the point that, while the solubility of the amine hydrochloride generally sets the maximum obtainable concentration for a given amine, the solubility of the free base and the pKa determine the maximum pH at which formulation as a solution is possible (assuming that the desired concentration exceeds the free base solubility). Shifting the pH–solubility profile to higher pH values for formulation purposes may require increasing the solubility of the free base. This increase might be accomplished by using an appropriate cosolvent. Since the dissociation characteristics of carboxylic acids and other acidic organic species are similar to those of organic hydrochlorides, it is expected that the pH–solubility profiles

of these organic acids, although reversed, can be characterized theoretically using the same treatment.

Several reports showed that the structure of an organic salt-forming radical influences the solubility of the resulting salt. The water solubilities of 16 salts (carboxylates, sulfates, sulfamates, and phosphates) of the weak base erythromycin were dependent on the size of the alkyl group of the acid (130). In a study with *N*-alkylsulfamates of lincomycin (66), a similar phenomenon was observed: solubility of these salts decreased as the size of the alkyl group attached to the acidic function increased.

Senior (131), in a study on the formulation and properties of the antibacterial chlorhexidine, determined the water solubilities of 35 salts and the free base. He found that inorganic salts had remarkably low solubilities while those of the lower aliphatic acids proved to be somewhat more soluble. Hydroxylation of the acid increased solubility, since salt formation with polyhydroxy acids, particularly the sugar acids, conferred extensive water solubility to the molecule.

Several investigators reported the influence of the solubility of a drug on its formulation and subsequent availability from the dosage form. In a discussion of the preparation and formulation of epinephrine salts in an aerosol system using liquefied gas propellants, Sciarra *et al.* (132) pointed out that the solubility characteristics of the agent are important in two respects. First, the solubility of the therapeutically active ingredient in the various propellants is an important consideration if the product is to be used for either local action in the lungs or systemic therapy. Second, the solubility of the drug in extracellular fluids plays an important role in selection of the compound. The bitartrate, malate, maleate, and fumarate salts of epinephrine were prepared and subjected to solubility and stability studies. While all salts had similar partition coefficients, the solubility of the maleate in several propellants and its stability in formulated aerosols made it the drug form of choice.

Ephedrine hydrochloride was more rapidly released than the free base from theobroma oil suppositories containing different surfactants (133). This enhanced rate of release (dialysis) was ascribed primarily to the greater aqueous solubility of the hydrochloride, which solubilized faster from the oil-in-water emulsion, whereas the ephedrine alkaloid base tended to remain behind in the oil phase.

The solubility of the active ingredient in ointment bases can dramatically influence its diffusion properties. A study of salicylic acid and its sodium salt showed that the diffusion of both was very low from hydrophobic bases, whereas the solubility of the drug significantly affected the diffusion from hydrophilic bases. The more soluble sodium salicylate diffused much faster from these latter bases than did salicylic acid (134).

Additional references on the relationship of salt form and solubility are listed in Table V.

Table V—Additional References on Salt Form and Solubility

Topic	Reference
Influence of solubility on the rate of GI absorption of aspirin	135
Effect of dosage form upon the GI absorption rate of salicylates	136
Physical–chemical properties of polyene macrolide esters and their water-soluble salts	137
Isolation and reaction products of orotic acid and amines and their solubility in water	138
Solubility and stability of erythromycin salts	139
Studies on pharmaceutical preparations of orotic acid: water-soluble properties of orotic acid salts	140
Solubility of antibiotics in 24 solvents	141, 142
Solubility of antibiotics in 26 solvents	143

Organoleptic Properties—Modern medicine requires that a pharmaceutical formulation be efficacious, safe, stable, and acceptable to the patient. Of primary importance in the development of oral dosage forms is taste acceptability. This factor presents no major problems when the drug is to be administered as a tablet or a capsule and swallowed as a unit but is clearly a prominent factor in patient acceptability when it is to be administered as a liquid, chewable tablet, or lozenge.

Since taste is a chemical sense, a substance must be dissolved if it is to elicit a taste sensation—either by taking it as a solution or by its dissolving in the saliva. Therefore, one method used to minimize undesirable organoleptic properties of pharmaceuticals involves the preparation of a poorly soluble salt form of the drug such that the saturation concentration is less than the taste threshold.

Erythromycin estolate (lauryl sulfate) has approximately one-twelfth the solubility of the free base, is tasteless, and is useful in the formulation of oral suspensions (144). A study on erythromycin salts showed that the bitterness level was dependent on two properties: (*a*) the water solubility of the salt, which is dependent on the size of the alkyl group attached to the acid function; and (*b*) the strength of the acid used to form the salt, *i.e.*, the stability of the salt (130). The stearyl sulfamate salt possessed the most desirable organoleptic properties.

Many problems concerned with formulation and stability of topical and oral pharmaceuticals containing bacitracin have been overcome by incorporating bacitracin into the formulation as its zinc salt. One distinct advantage over the parent compound is its lack of taste, caused by its relative insolubility. Thus, it is the preferred drug form for preparations where taste is a factor (145). Taste panel evaluations of the comparative bitterness of bacitracin zinc and bacitracin indicate that the taste of the zinc salt is more easily masked and that the presence of a bitterness-masking adjuvant, such as sucrose, increases the bitterness threshold ratio differences between the two compounds even further (146).

Propoxyphene napsylate, nearly water insoluble, is only slightly bitter to the taste as compared to the highly water-soluble hydrochloride (147) and can be readily formulated into a flavored aqueous suspension. The taste of these suspensions can be improved significantly by the addition of a common ion (sodium or calcium napsylate) to depress solubility further.

A newer approach to the improvement of drug palatability has been to form insoluble salts with ion-exchange resins. Several investigators described and tested the practical application of this method (148–150). Spross *et al.* (149) outlined the conditions necessary for improving the palatability of a drug by adsorbing it onto an ion-exchange resin without appreciably modifying its pharmacological effects. They found that: (*a*) the degree of drug release from the ion-exchange adsorbate depends on the

equivalent quotient between the electrolytes in the surrounding fluid and the ionic drugs, (*b*) the amount of ions is far less in the saliva than in the gastric juice (the temporary electrolyte contents can be estimated at 0.05 mEq in the saliva and at 10 mEq in the gastric juice), and (*c*) the exchange rates should allow the equilibria to be attained within a fairly short period. Insoluble drug resinates formed between dextran gel[2] cation exchangers and several basic drugs were in many cases much more pleasant tasting than their parent compounds. Furthermore, release of the drug from the ion-exchange adsorbate was quite rapid and complete under conditions prevailing in the GI tract.

Similar findings were reported using a polymethacrylic acid ion-exchange resin (150). In addition, coating the adsorbate particles with a 4:1 ethylcellulose–hydroxypropyl methylcellulose mixture further reduced bitterness. While *in vitro* release from the uncoated resinate was rapid and complete, release from coated adsorbates varied with the extent of coating.

Another approach to improving the taste properties of pharmaceutical agents is to prepare a pleasant-tasting soluble salt of a poor-tasting parent drug. This approach often can be very difficult, however, since solubilization of the parent compound usually imparts its unpleasant taste to the preparation. Nevertheless, some success has been reported using the artificial sweeteners cyclamate sodium and saccharin.

As described earlier, formation of *N*-cyclohexylsulfamate salts of several drug substances has produced better tasting derivatives with enhanced solubility properties (31, 32). The physicochemical and toxicological properties of benzalkonium saccharinate and a series of saccharinates of other quaternary ammonium compounds were reported (151). While conventional quaternary ammonium compounds have a very bitter taste, their saccharin analogs are sweet.

Potassium salts frequently possess an unpleasant taste and a metallic aftertaste. The palatability of some potassium salts in flavored vehicles was reported (152); while the salts had similar taste thresholds at effective therapeutic levels, all potassium salts exhibited inferior palatability.

Table III includes several samples of other salts that exhibit an improved taste relative to their free acid or base forms.

Stability—The chemical and physical stability of a pharmaceutical must be known, because it can influence the choice of dosage form, the manufacturing and packaging, and the therapeutic efficacy of the final preparation. Systematic determination of the thermal stability, solution stability (at various pH's), and light sensitivity of a drug and its derivatives, both alone and in the presence of common additives, provides essential input toward selecting the most suitable derivative and dosage form.

Depending on the route of degradation, different salt forms impart different stability characteristics to the parent drug by various mechanisms. Most commonly used are sparingly soluble salts which, when used in the formulation of suspensions, reduce the amount of drug in solution and, hence, its degradation. Differences in hygroscopicity of several salts influence the stability of the drug in the dry state. In some cases, the salt-forming radical itself enhances the stability of the parent agent.

The stability of penicillin G and its salts has been widely studied due to the drug's therapeutic importance and its characteristic instability. Schwartz and Buckwalter (153) described some of the stability characteristics of this antibiotic, stating that, with present techniques, a solution of penicillin cannot be made stable for more than 2 weeks, even at refrigerator temperatures. They also discussed the use of suspensions of sparingly soluble amine salts in aqueous vehicles as a means of "allowing marketing of a 'ready-made' penicillin product." Procaine, benzathine, and hydrabamine salts are marketed, and their acceptable stability as aqueous suspensions is based mainly on their insolubility and the minimization of degradation in solution.

A theoretical treatment of the solubility of these salts was presented in which equations were derived for calculating the solubility as a function of pH and the pH of minimum solubility (154, 155). These equations are based on the mass action law and its relationship to the ionization constants of the amine and the penicillin and the solubility of the salt in water. Since the salt in solution is partially dissociated, further suppression of the solubility may be achieved by the common ion effect. Swintosky *et al.* (156) demonstrated this effect with penicillin G procaine by adding procaine hydrochloride to the preparation and further enhancing its stability. The 8-chlorotheophylline salt (or complex) of penicillin G was reported to be water soluble, yet stable in solution (157). Since 8-chlorotheophylline is acidic, it has been postulated that a buffer effect could account for the stabilization of this "salt."

While penicillin G procaine is more stable in aqueous vehicles, it is less thermally stable than the sodium or potassium salts, decomposing if heated much above 60°. The sodium and potassium salts are known to withstand heating up to 100° for 4 days with little loss in potency (158). This behavior might well be due to differences in melting points—*viz.*, 106° for penicillin G procaine and ~215° dec. for the potassium salt.

Since hydrolysis of penicillin is dependent on moisture content, preparations in which moisture is rigorously excluded are quite stable in the dry state. A study on the effect of moisture on penicillin salts found the calcium salt to be less hygroscopic than the sodium salt and, hence, more stable in moist atmospheres (159). Similarly, penicillin G potassium is also much less hygroscopic than penicillin G sodium and has become the preferred form for marketing in the dry state (160).

Several studies reported the relative stabilities of thiamine salts, particularly the hydrochloride and the mononitrate (161–163). The mononitrate is observed to be less hygroscopic and is accordingly much less water soluble than the hydrochloride. Investigations of various preparations including compressed tablets, multivitamin capsules, and dry-filled vitamin B complex capsules at various temperatures showed that the mononitrate was more stable than the hydrochloride (164, 165). The stabilities of numerous thiamine salts were studied in aqueous solution and in dry powder preparations with various excipients (166, 167). In aqueous solution, the resulting pH was the chief factor controlling hydrolysis and oxidative decomposition of thiamine salts; their stability as powder

[2] Sephadex.

preparations was related to their aqueous solubility, with the sparingly soluble salts being more stable (and presumably less hygroscopic).

An orally administered drug must be stable in acidic solution because it generally must pass intact through the acidic environment of the stomach if it is to exhibit therapeutic blood levels. The advantage of erythromycin estolate over the free base lies in its low solubility in gastric juice, which enables it to be administered with food without any decrease in attained blood levels. The salt is more stable in the stomach because it remains undissolved. Therefore, it retains its potency even when exposed to acidic environments for extended periods (144).

In a study on the preparation and characterization of lincomycin cyclamate (33), it was noted that the cyclamate salt had an enhanced thermal stability over the hydrochloride. In a subsequent report (168), differential thermal analysis and thermogravimetric analysis showed that while the hydrochloride easily undergoes thermal degradation, the cyclamate anion confers a considerably greater thermal stability on the lincomycin moiety.

Mullins and Macek (169) showed that the physical and chemical stability of the calcium salt of novobiocin makes it the form of choice for the formulation of a liquid preparation of the antibiotic. The amorphous calcium novobiocin salt proved to be tasteless, yet fully biologically active and perfectly stable in aqueous suspension. Neither the sodium salt nor the free acid is suitable; the sodium salt cannot be formulated in a liquid due to its chemical instability, while the crystalline free acid is not absorbed from the GI tract. Amorphous novobiocin is absorbed but is metastable in solution and slowly converts to the unabsorbed crystalline form.

Other reports of alterations in stability characteristics due to salt formation are listed in Table VI.

Miscellaneous Properties—The salt form has been reported to influence other physicochemical properties of a drug substance. Studies illustrating the effect of the salt-forming radical on surface tension, deaggregation behavior, and ion-pair extraction have appeared.

The influence of the anion on the absorption processes of two charged species, dextromethorphan and tetracycline, was studied in the rat stomach (186, 187). A linear relationship existed between the rate of absorption from buffer solutions of the anions under investigation and their surface tensions. Thus, the absorption process was related to the surface activity of the various salts and not to their lipid solubilities. This change of surface activity with the buffer (or salt) species is similar to the results reported in a study of the surface activity of various phenothiazine salts (188).

The antibacterial chlorhexidine possesses surface activity. A study of the colloidal properties of some chlorhexidine salts showed that the counterion can affect the critical micelle concentration of a surface-active agent, and this effect was usually associated with a change in micellar size (189).

The deaggregation behavior of a relatively insoluble acid and its sodium salt was studied, and deaggregation was postulated to be a possible rate-limiting step in the absorption of a drug from a dosage form (190). While no direct comparisons of the two forms were made, inspection of the data shows that the deaggregation rate of the salt was considerably more rapid than that of the free acid in equivalent dosage forms. Therefore, if absorption is dependent on the dissolution rate, which in turn is dependent on the deaggregation rate, the salt should produce the highest and earliest blood levels. On the other hand, it is possible that hygroscopic (and deliquescent) salts can absorb atmospheric moisture, cause a sticky surface, and inhibit deaggregation.

Table VI—Additional References on Salt Form and Stability

Topic	Reference
Stability of chlorhexidine solutions	170
Stability of chlorhexidine when autoclaved	171
Anhydrotetracycline and 4-epianhydrotetracycline in marketed tetracycline and aged tetracycline products	172
Solid-state stability of some crystalline vitamin A compounds	173
Physicochemical studies on the stability of penicillin salts	174
Light sensitivity of tetracyclines	175
Hygroscopic properties, thermostability, and solubility of oleandomycin salts	176
Stability of orotic acid and its amine salts in aqueous solution	177
Some factors influencing the stability of tablets (aspirin)	178
Stability of aqueous solutions of sodium aminosalicylate	179
Hygroscopic properties of various preparations of erythromycin	180
Physicochemical studies on the decomposition of aminosalicylic acid and its salts	181
Stabilities of aqueous solutions of 2-diethylaminoethyl-3-methyl-2-phenylvalerate hydrochloride and its methobromide	182
Investigation of some properties of penicillin G salts	183
Stability of ferrous iron tablets on storage	184
Stability of aspirin aluminum compounded with antacids	185

Higuchi and coworkers presented an extensive study on the physicochemical basis of the ion-pair extraction of pharmaceutical amines. Distribution ratios of dextromethorphan (191) and chlorpheniramine (192) between an organic layer and water were highly dependent on the concentration and nature of the anion present. Less hydrophilic anions yielded more readily extractable ion-pairs. A study of the thermodynamic properties, enthalpy, free energy, and entropy, involved in the extraction equilibria of dextromethorphan ion-pairs indicated that the entropy change associated with transfer of the different anions between phases is the main controlling factor in the extraction process (193).

BIOAVAILABILITY

Most drugs prescribed in the United States are administered in solid and polyphasic dosage forms. Consequently, dissolution of the drug must precede the absorption process. The simplest model that adequately describes this process is shown in Scheme II.

$$\text{solid drug} \xrightarrow[1]{\text{dissolution}} \text{dissolved drug} \xrightarrow[2]{\text{absorption}} \text{drug in circulation}$$

Scheme II

Since the dissolution rate is generally slow for drugs with poor solubility, Step 1 is frequently rate limiting in the overall absorption process. As a result, the onset, intensity, duration of pharmacological activity, and, hence, bioavailability are affected by changes in dissolution rate. As discussed previously, administering a salt of the parent drug often proves to be an effective means of altering dissolution rate and absorption.

Table VII—Additional References on Bioavailability and Formulation Effects

Topic	Reference
Effects of various substances on the absorption of tetracycline in rats	197
Effects of dosage form upon the GI absorption rate of salicylates	136
Determination of *in vivo* and *in vitro* release of theophylline aminoisobutanol in a prolonged-action system	198
Ion-exchange resin salts for oral therapy: carbinoxamine	199
Latentiation of dihydrostreptomycin by pamoate formation	10
Solid-state ophthalmic dosage systems in effecting prolonged release of pilocarpine in the cul-de-sac	17
Absorption of erythromycin: various pharmaceutical forms	200
Comparative study of the absorption of drugs from old and new rectal preparations	201

Formulation Effects—Choice of the salt form of a drug may have a pronounced effect on the formulation of the parent compound. For example, Fenton and Warren (194) found there was no release of medicament from proflavine cream BPC, a water-in-oil emulsion containing 0.1% proflavine as the hemisulfate salt. They also investigated the release of various salts of proflavine with aliphatic carboxylic acids from water-in-oil cream emulsions. Salts formed with the water-soluble, oil-insoluble "lower" acids, such as formic and acetic acids, showed very poor release from a water-in-oil cream. The "higher" acid salts (*e.g.*, *n*-valeric, caproic, cyclohexanecarboxylic, and caprylic) all showed increased diffusion from similar emulsions since these salts are soluble in both water and oil. Their release was even greater from oil-in-water emulsions, however, in agreement with their preferential oil solubility. The *n*-valerate salt provided the most effective water-in-oil cream. The primary factor responsible for diffusion of proflavine from a water-in-oil cream is the low hydrophilic–lipophilic balance conveyed to the salt by the acid. This finding illustrates the desirability of carefully selecting the salt anion of a cationic drug in lieu of the nature of the dosage form.

Studies of the effect of formulation on the bioavailability of warfarin sodium relative to warfarin yielded interesting results (107, 195). Absorption of warfarin upon administration of the sodium salt as a lactose-base tablet was no better than that from a similar formulation of the free acid. In fact, absorption was further depressed when the salt was formulated with starch instead of lactose. Later results indicated that the *in vitro* water dissolution rate of a warfarin sodium tablet was 350 times that of a slowly dissolving warfarin tablet formulation, yet the latter exhibited rapid and complete absorption *in vivo*. The virtual insolubility of warfarin in acidic gastric fluids precluded its absorption from the stomach. However, the strongly acidic medium was necessary for tablet disintegration, which, in turn, was critical for absorption. Following initial exposure to 0.1 *N* HCl, *in vitro* dissolution of the warfarin tablet in pH 7.4 buffer was 14 times faster than that of the sodium salt, a result that explained the otherwise contradictory *in vivo* blood level data. Therefore, absorption was ultimately dependent upon gastric emptying rate and gastric pH, as long as the formulation disintegrated properly in the stomach.

The rectal absorption of aspirin, aspirin aluminum, and calcium carbaspirin from several suppository bases was studied in dogs (196). The absorption of aspirin aluminum from either cocoa butter or a polyethylene glycol base was poor. While the maximum salicylate levels produced by aspirin and calcium carbaspirin from the cocoa butter base occurred at a later time than from the other bases studied, minimal plasma levels were exhibited by a polysorbate 61 base formulation. The highest peak and largest area under the blood level curve were seen with calcium carbaspirin in a vegetable fatty acid glyceride base. The poor absorption of aspirin aluminum from suppositories was not unexpected since it is poorly soluble. Furthermore, as pointed out for aspirin aluminum tablets (105, 106), an insoluble aluminum compound may form on the surface of the dissolving drug, further impeding its dissolution rate and bioavailability.

Additional references on bioavailability and formulation effects are given in Table VII.

Absorption Alteration—Several years ago, clinicians claimed that certain salts of theophylline were therapeutically preferable to other salts or to the free acid (202–204). For example, Schluger *et al.* (204) found higher blood theophylline levels after administering uncoated tablets of theophylline ethylenediamine than were observed with enteric-coated tablets of choline theophyllinate. These results were at variance with the *in vivo* work of Gagliani *et al.* (202), who found that the oral ingestion of choline theophyllinate produced significantly higher blood levels than the ethylenediamine salt. This apparent discrepancy could be explained by the formulation effects of tablet coating, *etc.*, which was not discussed in the work of Gagliani *et al.* In another study, a slightly more rapid rise in blood concentration and a greater area under the curve were observed with theophylline isopropanolamine than with theophylline ethylenediamine (203). It was suggested that the difference was a result of the greater water solubility of the isopropanolamine salt.

The *in vitro* dissolution rates of the choline and isopropanolamine salts of theophylline have been observed to be three to five times greater than the ethylenediamine salt, depending on the dissolution medium (98). It has been suggested that these differences in dissolution rate are consistent with, and offer an explanation for, the clinical results.

In a comparative study of the absorption of ampicillin trihydrate and ampicillin potassium following oral administration (205), the potassium salt yielded 37% higher peak levels and a larger area under the curve. Only 36% of the administered ampicillin trihydrate was absorbed while 53% of the potassium salt was absorbed. Determining the percentage of each drug eliminated in the urine showed that 39% was eliminated following administration of the trihydrate and 52% from the potassium salt. The entire difference between the two drug forms was accounted for in the initial 4 hr postadministration.

Several studies compared blood levels obtained with erythromycin and its salts and esters. Erythromycin estolate produced blood levels that were severalfold higher than those obtained with erythromycin base or erythromycin stearate (206–208). These differences were found to persist in the fasting as well as nonfasting subjects (207), indicating that food did not appreciably alter the absorption of erythromycin estolate when given under single or

multiple dosing (207). This finding is explained by the fact that this salt is acid stable and alkaline dissociable, permitting its passage through the acid of the stomach both in fasting and nonfasting subjects (209). Accordingly, the antibacterial activity remained essentially the same when this form of erythromycin was given, regardless of the state of fasting of the subject (209).

On first inspection, the higher serum levels attained with erythromycin estolate suggest that the salt form is more readily absorbed. Also critical to efficacy, however, is the volume of distribution of the drug, since extensive binding to plasma proteins can render a drug unavailable for activity at the biophase. Therefore, the significance of blood level data greatly depends on the measure of the free or unbound fraction of total antibiotics in the blood, which more directly indicates probable therapeutic benefit.

Wiegand and Chun (210) showed that, despite the higher blood levels attained with erythromycin estolate (after correction for half-life differences), the stearate salt produced seven times more free drug in serum than did the estolate salt. This finding explained the higher total tissue levels observed on administration of the stearate. They attributed the difference to a greater serum protein binding of the intact erythromycin estolate, proving that its higher serum levels did not necessarily reflect more efficient absorption.

Marked differences have been observed following oral administration of various salts of penicillin. Penicillin G potassium has been compared with penicillin G benzathine (benzethacil) (211, 212), penicillin G hydrabamine (213), penicillin V (214), penicillin G procaine (212), and penicillin G ammonium (215). As anticipated, penicillin G potassium produced higher and earlier blood levels than penicillin G benzathine (211, 212). Furthermore, tablets of penicillin G potassium buffered with sodium citrate yielded higher peak levels than unbuffered penicillin G potassium. While the absorption of buffered tablets was apparently not significantly affected by food intake, the unbuffered tablets yielded lower average levels and irregular absorption under similar conditions.

When penicillin G potassium was compared with penicillin G hydrabamine (213), a less soluble salt, blood levels similar to those produced by penicillin G benzathine were observed. When equivalent doses were administered, the penicillemia that occurred with penicillin G hydrabamine was only one-third or one-fourth as great and was of shorter duration than that produced by penicillin G potassium.

Budolfsen *et al.* (212) found that peak concentrations of penicillin G potassium were three to four times those obtained with penicillin G procaine and five to six times those of penicillin G benzathine, indicating a more rapid rate of absorption. The therapeutic action was related to the maximum concentration attained, but it also depended on the persistence of penicillemia, which was greater with penicillin G potassium than with the other two compounds. The authors suggested that the lower blood levels attained with the relatively insoluble penicillin G benzathine were caused by its destruction in the GI tract prior to absorption. This suggestion seems unlikely, however, since the drug should not degrade very rapidly as an undissolved suspension.

Because of its superior stability in gastric juice, penicillin V produces higher blood penicillin levels than corresponding doses of penicillin G. As a result, extensive investigations have been conducted with various salts of penicillin V (99, 214–220). For instance, tablets, capsules, and oral suspensions of penicillin V acid produced significantly higher blood concentrations than comparable penicillin G preparations (214). In the same study, the average serum levels produced by the benzathine salt of penicillin V were significantly higher than comparable doses of penicillin G benzathine for the first 4 hr but lower thereafter, again illustrating the value of using an insoluble salt to prolong blood levels of an acid-unstable compound. In another study (215), penicillin V acid was shown to produce higher and more prolonged plasma concentrations than either penicillin G potassium or ammonium, whose properties were comparable.

Plasma levels were correlated with dissolution rates of various forms of penicillin V (99). As solid dosage forms, the readily soluble potassium and calcium salts produced earlier and higher blood levels in fasting subjects than either the free acid or its benzathine salt. On the other hand, when the potassium and benzathine salts were administered orally as solutions, absorption was the same, implying that the poor solubility of the benzathine salt was responsible for the inferior blood levels obtained from its solid dosage forms. Other studies found that, while the potassium salt is 40% better absorbed than the free acid in fasting subjects, both forms produce therapeutic levels when administered with food (218).

Experiments with fistulated dogs indicated that penicillin V is absorbed primarily from the stomach. Therefore, it is not surprising that the potassium salt should show higher blood levels on oral administration since it is the most soluble salt in gastric pH (216). In accordance with this observation, it was also reported that the benzathine salt exhibited higher serum levels in patients with gastric achlorhydria (pernicious anemia) than in patients with normal gastric function (219). Differences in gastric emptying time may also explain this result.

Several studies compared the absorption of tetracycline and its salts (103, 221, 222). For example, serum concentrations in dogs and humans showed that a phosphate complex salt of tetracycline was absorbed more rapidly and gave higher blood levels during the first 6–8 hr than did tetracycline hydrochloride (221). The total amount of drug absorbed was about twice as great with the former compound.

Another study (222) suggested that tetracycline base produced higher blood levels than tetracycline hydrochloride. However, a subsequent investigation (223) showed that, in the absence of adjuvants or fillers, tetracycline hydrochloride and tetracycline base were absorbed equally well. Results obtained in this same study indicated that tetracycline hydrochloride encapsulated with citric acid produced higher serum concentrations than tetracycline hydrochloride mixed with hexametaphosphate or the phosphate complex salt of tetracycline.

In a study comparing urinary excretion rates and *in vitro* dissolution, the absorption of tetracycline and tetracycline phenolsulfonaphthaleinate was rate limited by their dissolution rates, whereas tetracycline hydrochloride absorption was rate limited by the absorption process itself (103).

Several lincomycin salts were studied for their comparative availability (224). In particular, the blood levels obtained with the relatively water-insoluble hexadecylsulfamate salt were compared to those of the hydrochloride following oral administration. Higher and extended whole blood and serum concentrations were obtained in mice, rats, and dogs with the hexadecylsulfamate. However, subcutaneously administered lincomycin did not produce significantly different fractions absorbed, regardless of which salt was administered. It is not known whether the greater area under the curve with oral administration of lincomycin hexadecylsulfamate is due to greater absorption from the GI tract, slower renal clearance, or greater enterohepatic circulations.

Salts of streptomycin, neomycin, viomycin, and streptothrycin have been formed with: (*a*) polyacrylic acids, (*b*) sulfonic or phosphorylated polysaccharides, and (*c*) natural polycarboxyl acids from a series of polyuronic substances and polysaccharide derivatives containing carboxyl groups (18). The report indicated that these salts were absorbed from the injection locus primarily by the lymph system. Blood levels from the salts were generally lower but were maintained for a longer time than the equivalent amount of antibiotic alone, and higher concentrations of longer duration may actually be produced in the lymphatic drainage.

The influence of salt formation on the onset and duration of pharmacological activity also was illustrated with tolbutamide and several of its salts (101). Following oral administration, the sodium salt produced a rapid decrease in blood sugar level followed by a rapid recovery. By contrast, the free acid of tolbutamide caused a slow and prolonged drop in blood sugar level, a preferred effect since the chance of hypoglycemic shock would be lessened. This finding also illustrates the often overriding influence of the actual disease state on the choice of drug form.

Additional references on the implications of salt formation on absorption are listed in Table VIII.

Pharmacokinetics—Because of the various new properties that are usually imposed on a compound by salt formation, the pharmacokinetics of the drug necessarily change as a function of these properties.

For example, a pharmacokinetic evaluation comparing ampicillin sodium and potassium with ampicillin trihydrate was performed after oral administration to beagle dogs (243). The absorption rate constants of the sodium and potassium salts, which were similar, proved significantly greater than the rate constant of ampicillin trihydrate, resulting in earlier, higher peak concentrations with two to three times higher serum concentrations during the 1st hr. Yet, any differences between the fraction absorbed for the three products were not statistically significant. Apparently, although dissolution of the ampicillin trihydrate was the rate-limiting step in its absorption, the overall extent of bioavailability remained unaffected.

An interesting study of the biliary excretion of erythromycin base and erythromycin estolate was reported (244). The biliary excretion of erythromycin base was high, while that of erythromycin estolate was much lower; preferential secretion of erythromycin in the bile could partially account for the lower serum levels exhibited by the base. However, the proportion of the ingested dose secreted in the bile was small, and the total amounts involved were not sufficient to account entirely for the differences in serum concentrations attained. Undoubtedly, the protein binding studies of Wiegand and Chun (210) (discussed under *Absorption Alteration*) more satisfactorily explain the difference in serum concentrations.

Table VIII—Additional References on Bioavailability and Absorption Alteration

Topic	Reference
Blood levels produced by three theophylline-containing elixirs	225
Naproxen oral absorption characteristics	226
Effect of food on absorption of a new form of erythromycin propionate	227
Effect of the anion on the absorption of tetracycline from the rat stomach	186
Blood levels following oral administration of different preparations of novobiocin	228
Absorption of iopanoic acid and its sodium salt	229
Oral absorption of secobarbital (quinalbarbitone) and its sodium salt	230
Absorption rate of barbiturates in humans	231
Morphine and atropine mucate	232
Excretion of bephenium salts in urine of human volunteers	233
Polymethylene bis(isothiuronium) salts: antituberculosis activity	234
Prolonged antitussive action of a resin-bound noscapine preparation	235
Pharmacology of sulfapyridine and sulfathiazole	236
Evaluation of plasma concentrations of propoxyphene utilizing a hybrid principal component analysis of variance technique: equimolar doses	237
Antrycide, a new trypanocidal drug	238
Pralidoxime methanesulfonate: plasma levels and pharmacokinetics after oral administration to humans	239
Intestinal absorption of pralidoxime and other aldoximes	240
Blood plasma levels and elimination of salts of pralidoxime (2-PAM) in humans after oral administration	241
Enhancement of GI absorption of a quaternary ammonium compound by trichloroacetate	242

Often, salt formation can be used to modify drug absorption and dose tolerance favorably. For example, aminosalicylic acid exhibits a short half-life and, therefore, requires large and frequent doses which may cause gastric irritation. Consequently, different chemical forms such as salts have been prepared (119, 245–247) to reduce the incidence of gastric irritation, increase absorption, and prolong blood levels.

Aminosalicylic acid is an interesting example in other ways; considerable confusion about this drug exists because many fail to recognize its nonlinear pharmacokinetics. Several definitive studies were reported regarding the absorption of the acid and its sodium, potassium, and calcium salts from solution, suspension, and tablet formulations (245, 246). Comparison of the relative bioavailabilities of aminosalicylic acid suspended in water and its salts dissolved in water showed that, while differences in rate of absorption were found to exist, absorption of both the acid and its salts was essentially complete. Absorption of the free acid from tablets reached only 77% of the dose, whereas that of the tableted salts was rapid and complete.

Regardless of formulation, the area under the plasma concentration–time curve of unmetabolized drug from free acid administration was less than that for the salts. This result was attributed to concentration-dependent metabolism during absorption: when the rate of absorption is high, the metabolic processes become saturated and more unmetabolized drug remains in the blood; conversely,

Table IX—Additional References on Bioavailability and Pharmacokinetics

Topic	Reference
Pharmacodynamics of fosfomycin (phosphonomycin) after intravenous administration to humans	248
Pharmacodynamics of phosphonomycin after oral administration to humans	249
Comparative studies on distribution, excretion, and metabolism of ^{3}H-hydroxyzine and its ^{14}C-methiodide in rats	250
Pharmacokinetics of ampicillin trihydrate, ampicillin sodium, and dicloxacillin sodium following intramuscular injection	251
Physiological disposition of fenoprofen in humans: pharmacokinetic comparison of calcium and sodium salts administered orally	252

when the absorption rate is low, as for the free acid, a higher percentage of drug is metabolized.

Additional references regarding bioavailability and pharmacokinetics are presented in Table IX.

GENERAL PHARMACY

Pharmacological Effect—Chlorpromazine hydrochloride and quaternary chlorpromazine chloride were investigated with respect to their effects on the central nervous system (CNS) (253). The quaternized compound was less potent and more toxic in rodents than the parent tertiary compound.

Naloxone, an effective opiate antagonist, is generally used as the hydrochloride salt; however, the drug has a very short duration of action. The mucate salt was prepared to extend its duration of action, since mucic acid is only slightly soluble in water (254). In a test on the blocking of morphine activity in mice, however, the mucate salt did not differ in duration from the hydrochloride. These investigators assumed the same receptor site for naloxone as for morphine and, since Heron's (232) work suggested that the receptor had a greater affinity for morphine mucate than for the free base, it also should have a greater affinity for naloxone mucate. The results disproved this hypothesis. Furthermore, this theory implies that intact salt reaches the receptor, which is highly unlikely, regardless of whether the drug is administered as a solution or as a suspension.

A series of salts of 9-aminoacridine and its derivatives was prepared and screened for antifungal and antibacterial activity (255–257). By using salts of fatty acids, the antifungal action was found to parallel the length of the carbon chain of the anion, with maximal activity occurring with acridine caproate, undecylate, and undecylenate (where undecylenic acid also exhibits some intrinsic antifungal activity) (255). This result appears reasonable, because these salts would be more lipid soluble and could be expected to pass through the cell wall of the infecting organism more readily, probably as an ion-pair.

The efficacy of bases or salts as topical anesthetics for relieving cutaneous itch, burning, and pain in unbroken skin has also been examined (258). In these experiments, itching and pricking were induced by an alternating current of low amperage and voltage applied to the skin or by exposure of the skin to UV light. Interestingly, aqueous solutions of salts of the local anesthetics did not alleviate itching or burning in any of the subjects, although saturated solutions of their bases in a mixture of water, 40% alcohol, and 10% glycerol were claimed to be effective. Such transport phenomena across the stratum corneum are often dependent on the polarity of the drug and vehicle and on the binding of the drug to keratin.

Table X—Additional References on General Pharmacy and Pharmacological Effect

Topic	Reference
Differential excretion of bromide and chloride ions and its role in bromide retention	259
Pharmacological study of calcium methionate	260
Synthesis and *in vitro* fungistatic evaluation of some *N*-substituted amides and amine salts of sorbic acid	261
Antiamebic studies on clamoxyquin [5-chloro-7-[[(3-diethylaminopropyl)amino]methyl]-8-quinolinol] *in vitro* and in experimentally infected animals	262
Adjunctive value of oral prophylaxis with the oximes pralidoxime (2-PAM) lactate and pralidoxime methanesulfonate to therapeutic administration of atropine in dogs poisoned by inhaled sarin vapor	263
Pralidoxime (2-hydroxyiminomethyl-*N*-methylpyridinium) methanesulfonate and atropine in the treatment of severe organophosphate poisoning	264
Efficacy and limitations of oxime–atropine treatment of organophosphorus anticholinesterase poisoning	265
Antitussive activity of enoxolone (glycyrrhetinic acid) and its derivatives	266
Pharmacological properties of glycyrrhetinic acid hydrogen succinate (disodium salt)	267
Ganglionic blocking activity of diastereomeric dimethylaminobornyl acetates and their methiodides	268
A new potent nonnarcotic antitussive, 1-methyl-3-[bis(2-thienyl)methylene]piperidine: pharmacology and clinical efficacy	269

Additional references on pharmacological effects can be found in Table X.

Dialysis—Dialysis through a cellophane membrane of the hydrochloride or sodium salts has been studied with several drugs (270). In many cases, it appeared that the ionic form of the drug was bound to the membrane whereas the nonionized form was not. Ephedrine hydrochloride presented an interesting example, however, since it dialyzed considerably faster than its corresponding base. It was theorized that the chloride ion dialyzed rapidly, enhancing the rate of dialysis of the ephedrine ion. Accordingly, when chloride ion was present on both sides of the membrane, the observed rate of dialysis for the ephedrine ion was comparable to the ephedrine base.

The diffusion of sodium chloride through a lipoprotein interface was very slow, especially if calcium chloride was present on both sides of the interface (271). In the presence of low concentrations of choline chloride or carbamylcholine chloride, the diffusion of sodium chloride is more rapid. Apparently, choline salts are able to increase the permeability of the lipoprotein to salts, which may relate to the physiological action of choline salts.

Miscellaneous—Release rates were determined for aminophylline, ephedrine alkaloid, and ephedrine hydrochloride from theobroma oil suppositories containing nonionic surfactants (133). While surfactants with hydrophilic–lipophilic balance (HLB) values less than 11 only minimally affected release rate, rates increased with surfactants of HLB values greater than 11. Under optimal conditions, aminophylline was faster than ephedrine hydrochloride which, in turn, was superior to the ephedrine base.

Willis and Banker (272) reported on the formation of polymer–drug salts as an approach to the physicochemical design of dosage forms. Poly(methyl vinyl ether/maleic

anhydride) salts of methapyrilene were prepared and tested with the free base for *in vitro* dissolution and dialysis. Their dissolution and dialysis rates were not appreciably different from the free drug or its hydrochloride salt. Various poly(methyl vinyl ether/maleic anhydride) hemiester salts of methapyrilene exhibited substantially slower release than the polymer ether salts, hydrochloride salt, or free base forms. Polymer–drug salts thus appear to have promise.

A series of metallic salts of edetic (ethylenediaminetetraacetic) acid were tested *in vitro* to determine their effect on blood coagulation (273). The results showed that only the dipotassium and disodium salts had any effect on coagulation. It was theorized that the lack of anticoagulant activity resulted from an almost complete suppression of ionization of the heavy metal salts.

Interesting research regarding the angina-preventive effect of some chromone-2-carboxylate salts showed a direct correlation between biological activity and pKa of the salt-forming amines (274).

Lin *et al.* (112) investigated the relationship between salt form and biological activity of a given antihypertensive. While the intrinsic dissolution rates of the dihydrochloride and disulfate salts were many fold greater than the monohydrochloride, the hypotensive potencies of the salts did not differ significantly from one another in an anesthetized dog study. Yet, when administered to renal hypertensive dogs, the dihydrochloride and disulfate salts produced greater hypotensive effects than did the monohydrochloride.

TOXICOLOGICAL CONSIDERATIONS

Toxicity of Salt Ion—Any discussion regarding the toxicity of salts of a drug must consider the pharmacological properties of the cation or anion used to form the salt as well as those of the free drug, since any of these may produce toxic effects. The toxicology of several ions that are commonly used to form salts and that are relevant to this review were discussed in depth (275).

Toxicity from ingestion of calcium salts of drugs is rare. If hypercalcemia occurs, however, calcium deposits in the kidney can bring on a reduction of renal function. The principal toxic effects of lithium also involve the kidneys. When small amounts of lithium are taken, no apparent damage occurs; yet large amounts of the metal can lead to irreversible damage. An apparent correlation was observed between lithium dosage and sodium intake (276). When lithium dosage was low or sodium intake was high, rats were able to excrete all lithium given and sustained a reversible polyuria. Conversely, if large amounts of lithium were administered to the rats or if their sodium intake was lowered, they incurred irreversible kidney damage. Ammonium ion, although it serves a major role in maintenance of the acid–base balance of the body, can be toxic in high concentrations and initiate CNS derangements.

Sulfate ions given orally tend to be minimally absorbed and may act as a laxative. The nitrate ion is irritating to the GI tract, causing nausea and gastric distress. Also, intestinal bacteria may convert the nitrate ion to nitrite which oxidizes hemoglobin to methemoglobin. The citrate ion, an intermediary in carbohydrate metabolism, can form a soluble complex with calcium which is poorly dissociable and rarely causes any toxic reactions. While tartrate ions are usually absorbed minimally from the GI tract, high concentrations reaching the circulation can cause renal damage.

Acetate and lactate ions are normal metabolites and appear to be well tolerated in relatively large amounts. Iodide and bromide ions can produce conditions known as iodism and bromidism, respectively. Bromide intoxication occurs quite frequently, since bromides are used as ingredients in some nonprescription preparations (277–280). Bromide is slowly excreted by the kidney (its half-life is 12 days) and tends to accumulate when taken for prolonged periods or when used by patients with decreased renal function (277).

Toxicity of Salt Form—Provided the salt-forming agents are nontoxic, the relative toxicities of a series of salts of a compound are often observed to reflect directly their absorption rates. For example, the toxicities of dibromide, dichloride, diiodide, and dimethylsulfate salts of quinapyramine[3], a trypanocidal drug, were determined (238). The sparingly soluble halogen salts were much less toxic subcutaneously or intramuscularly than the freely soluble dimethylsulfate, yet all salts showed about equal toxicity upon intravenous administration. The difference in toxicities obviously resulted from rapid absorption of the methylsulfate compared to the slowly absorbed, poorly soluble halogen salts. Similar reasoning has been used to explain the acute oral toxicity of propoxyphene hydrochloride in rodents, which is twice that of equimolar doses of the napsylate salt (281).

Several salts of benzphetamine and etryptamine were prepared as potential sustained-release formulations (114). The water solubility, *in vitro* dissolution rates at pH 1.0 and 7.2, and the median lethal times (LT_{50}) were determined for each salt. Both the LT_{50} and LD_{50} (determined on only a few salts) increased as the *in vitro* dissolution rate at pH 7.2 decreased. While dissolution at pH 1.0 did not correlate well with toxicity, the LT_{50}'s were inversely related to the square root of the dissolution rates at pH 7.2.

Toxicity studies comparing iopanoic acid, a cholecystographic contrast medium, with its sodium salt (115) showed that the salt form has 10-fold greater toxicity. The LD_{50}'s of the free acid and the salt were 22 and 2.32 g/kg, respectively. It was postulated that the free acid precipitated from the sodium salt upon its reaction with gastric hydrochloric acid. The fine, amorphous particles of precipitated acid had a greatly increased surface area and, therefore, dissolved more rapidly than even fine crystals of the free acid. The faster and more complete drug absorption then resulted in increased toxicity.

Salts exhibiting greater water solubility than their parent compounds or less soluble salts are not always more toxic. For example, various inorganic and organic ions were used to prepare salts of methyl pyridinium-2-aldoxime that would have greater water solubility and would eliminate undesirable side effects due to the iodide ion (95). Even though the aqueous solubility of the majority of these salts was many times greater than the iodide, their toxicity on a molar basis was not significantly different, with the exception of the dihydrogen phosphate salt which was 15%

[3] Aritrycide.

Table XI—Additional References on Toxicological Considerations

Topic	Reference
Toxicity and absorption of 2-sulfanilimidopyridine and its soluble sodium salt	285
Sorbic acid as a fungistatic agent for foods: harmlessness of sorbic acid as a dietary component	286
Toxicity and distribution of erythromycin	287
Further toxicological studies with erythromycin	288
Pharmacology and toxicology of erythromycin estolate	289
Erythromycin propionate (propionylerythromycin): a review of 20,525 case reports for side-effect data	290
New class of antibiotic salts of reduced toxicity	22
GI intolerance to oral iron preparations	291
Comparative toxicology of iron compounds	292
Influence of the dissolution rate of lithium tablets on side effect	123
Toxicity and tissue distribution studies on the hydrochloride, bismuth iodide complex, and a resinate of emetine	293
Bacitracin zinc in pharmaceutical preparations	145
New approach to quaternary ammonium compounds	151
Pharmacology of choline theophyllinate	294

more toxic. Further research with oximes revealed that other salts are also toxic (282).

GI bleeding is a common toxic effect of aspirin for a large percentage of the population. Consequently, a search was initiated for an aspirin derivative that would not induce GI blood loss (283). All compounds prepared, however, including the sodium and calcium salts, caused GI hemorrhage with a severity similar to aspirin.

Polyene antibiotics are potent antifungal agents but bear considerable toxicity. Even though the methyl ester hydrochlorides of these compounds are more soluble, they retain almost all of their antifungal activity and, more significantly, show a uniform decrease in toxicity compared to their parent compounds (284).

Additional references on toxicological considerations of salt formation are given in Table XI.

CONCLUSIONS

Salt formation is a means of altering the physical, chemical, and biological characteristics of a drug without modifying its chemical structure. Clearly, the salt form can have a dramatic influence on the overall properties of the parent compound. At present, selecting a salt form that exhibits the desired combination of properties is a difficult semiempirical choice. Pharmaceutical scientists now recognize these facts and are beginning to study the effects of different salt forms on the physicochemical properties, bioavailability, and toxicity of drug substances.

Although now only a few generalizations are available to predict the effect of particular salt forms on the characteristics of a drug, perhaps in time it will be possible to evolve increasingly more powerful generalizations regarding the effect of a salt on the properties of its parent compound. In addition, we predict that polymer–drug salts will have a revolutionary effect on future trends in drug therapy, particularly in the areas of reducing drug toxicity and in controlling the release profile of novel drug delivery systems.

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ˣ To whom inquiries should be directed.

Exhibit F

cillin G (Na) in methanolic HCl. In this case, in addition to the penicillin G zone, two other spots were detected. One of these (R_f 1.12 in System *A*) corresponded to the methyl α-D-penicilloate spot obtained with solutions in absolute methanol, while the substance giving the other unidentified spot (R_f's: *A*, 0.49; *B*, 0.28) is presumably benzylpenillic acid (II) which is the normal rearrangement product of benzylpenicillin in acid solution. Spots corresponding in R_f values to II have been detected in deteriorated procaine penicillin G solutions in water.

The TLC procedures described should be applicable to the detection of procaine and its degradation products in the presence of other drugs which are formulated with procaine, *e.g.*, epinephrine, ephedrine, morphine, scopolamine, paromomycin, dihydrostreptomycin. Since these latter compounds and penicillin G do not give color reactions with the Bratton-Marshall reagent (12), it should be possible to use this reaction as the basis for the assay of procaine in the presence of these compounds. Although the Bratton-Marshall reagent has been used for the assay of procaine penicillin G (13), the method appears to have been largely neglected by other workers. The usefulness of this reagent for the assay of procaine is presently being assessed in these laboratories.

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Pharmaceutical Heterogeneous Systems IV: A Kinetic Approach to the Stability Screening of Solid Dosage Forms Containing Aspirin

H. V. MAULDING, M. A. ZOGLIO, F. E. PIGOIS, and M. WAGNER

Abstract □ The effect of common tablet excipients and physiologically active materials on the hydrolysis of aspirin in aqueous suspension has been studied. The relationship of hydrolytic rate to solvent concentration in these systems has been determined. The results obtained have been utilized in the stability ranking for various tablet mixes. The kinetic ranking is compared to the relative stability of tablets and powders. An attempt is made to correlate extrapolated kinetic data with stability results for the tablets.

Keyphrases □ Heterogeneous systems—pharmaceutical □ Aspirin dosage forms—stability screening □ Stability, aspirin dosage forms—kinetic ranking □ UV spectrophotometry—analysis □ Colorimetric analysis—spectrophotometer

Prior investigations have shown an inverse relationship between aspirin stability and the presence of moisture at ambient or somewhat higher temperatures although secondary phenomena as the pH of the water may alter the rate of degradation (1–6). The deleterious action of fatty acid lubricants on acetylsalicylic acid has been related to hydrolysis (4). It has been previously mentioned that one may compare an aspirin tablet to an aqueous suspension—approaching but not attaining zero water concentration—in order to facilitate explanation of this solvolytic degradation (3). However, the complexities rendered by the multiplicity of variables in heterogeneous systems of this nature make interpretation rather more difficult than in, for example, solution or homogeneous kinetics where the components and species can be accounted for in totality in many cases.

Increased rates of aspirin decomposition in the presence of several excipients and antacids have been reported several times in the literature (1, 3, 4, 7, 8). These agents reported were found to markedly accelerate salicylic acid formation.

More recently Guttman (9) has observed considerable degradation of buffered aspirin tablets in chloroformic solutions and this breakdown is apparently not proportional to water content of the chloroform.

Garrett suggested the possibility that normally first-order decomposition processes may become zero-order in saturated solutions (6) and this has been corroborated by others (3).

The purpose of this study was the evaluation of the effect of some commonly used tablet additives regarding their influence on the rate of generation of salicylic acid from aspirin.

Table I—Relative Stability Data for Acetylsalicylic Acid in Tablets and Powders[a,b]

Mix No.	Additive	Mg. Salicylic Acid[c] Formed/200 mg. Aspirin in Sample	Relative[d] Ranking
	Tablets		
C	10% Hexamic acid	0.38	1
F	None	0.79	2
A	10% Aluminum hydroxide	2.03	5
B	5% Calcium stearate	4.73	12
E	5% Magnesium stearate	5.93	15
D	10% Magnesium trisilicate	38.43	100
	Powders		
C	10% Hexamic acid	0.44	1
F	None	0.84	2
A	10% Aluminum hydroxide	2.17	6
B	5% Calcium stearate	4.65	13
E	5% Magnesium stearate	5.98	16
D	10% Magnesium trisilicate	36.60	100

[a] Time, 45 days, temperature, 40° (±0.25°). [b] Stored in 30-ml. vials sealed with rubber closures and aluminum crimp tops. [c] Salicylic acid formation corrected to 1% moisture for all mixes. [d] Ranking based on 100 for *D* with regard to salicylic acid formation and is only relative.

For these comparisons aspirin powder mixes, tablets and suspensions were utilized in which a standard-type aspirin formulation was employed along with 5 and 10% of the various excipients and antacids examined.

EXPERIMENTAL

Powder Mix—A basic powder mix containing the following relative amounts of ingredients was prepared: aspirin (40 mesh), 200 mg.; microcrystalline cellulose,[1] 25 mg.; corn starch, 24 mg.; stearic acid USP, 11 mg.; lactose (spray-dried), 10 mg.; total, 270 mg.

The basic mix (Mix *F*, Table I) was stirred for 20 min. on a mixer (Hobart) and 2% by weight of water added at a rate of 1 ml./min. until it was thoroughly mixed with the other constituents. To this powder was separately added 27 mg./270 mg. basic mix or 10% of each of three substances: (*a*) aluminum hydroxide; (*b*) magnesium trisilicate; and (*c*) hexamic acid and 13.5 mg./270 mg. basic mix of (*a*) calcium stearate and magnesium stearate. Weighed samples of these powders were sealed in 30-ml. multiple-dose vials with rubber closures and aluminum crimp tops. These powder samples were stored at 40° and samples periodically removed for assay of salicylic acid. Moisture content for mixes and tablets were determined by the Karl Fischer procedure.

Tablet Preparation—Portions of the above mixes were tableted on a Stokes E machine using a 10-mm. standard punch to give 300-mg. tablets. No attempts were made to maintain a constant hardness due to difficulties with flow of some mixes. The tablets were stored in 30-ml. vials under the same conditions as the powder mixes and sampled after 45 days for aspirin breakdown.

Free Salicylic Acid Determination—Samples of powder mixes or tablets were carefully and completely removed from the 30-ml. multiple dose vials in which they were stored. These samples were ground thoroughly in a mortar and a quantity equivalent to 200 mg. aspirin, along with 100 mg. citric acid dissolved in 10 ml. water-saturated chloroform with agitation (1 min.). This solution was

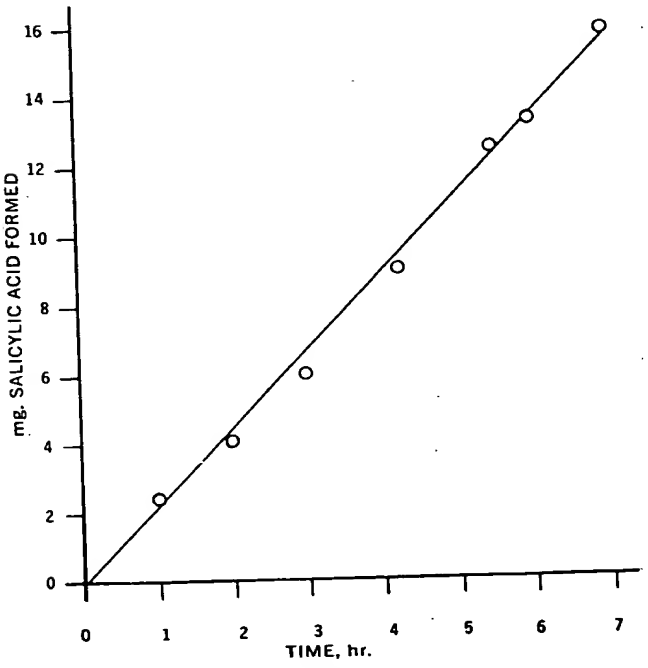

Figure 1—*Apparent zero-order plot for aspirin hydrolysis at 40° in aqueous suspension containing 6 ml. water and 594 mg. Mix A (samples equivalent to 400 mg. aspirin). 10% aluminum hydroxide is the additive.*

[1] Avicel, American Viscose.

Table II—Relative Rate Data for Salicylic Acid Formation from Powder Mixes in Suspension

Mix No.	Additive	Mg. Salicylic Acid[a,b] Formed per Hour	Relative[c] Ranking
F	None	0.054	1
C	10% Hexamic acid	0.075	1+
A	10% Aluminum hydroxide	0.329	6
B	5% Calcium stearate	0.848	16
E	5% Magnesium stearate	0.978	18
D	10% Magnesium trisilicate	2.31 (5.70)[d]	43 (105)[d]

[a] The apparent zero-order rate constant for a one-ml. sample at 40° (±0.2°). [b] Sample used equivalent to 400 mg. aspirin. [c] Relative ranking based on *F* as 1. [d] The apparent zero-order rate constant was calculated for a 0.1-ml. sample and corrected to 1 ml. assuming saturation did not occur at higher volumes (0.5 ml.; Fig. 4).

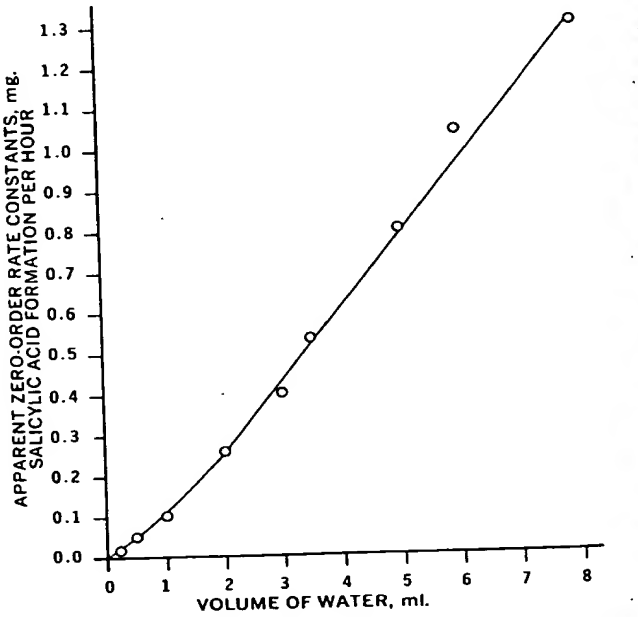

Figure 2—*Relationship of salicylic acid generation to the volume of water contained in Mix A suspensions. 10% aluminum hydroxide is the additive. Temperature, 40°.*

poured onto a column of 8 g. acid-washed diatomaceous earth[2, 3] previously mixed with 2% ferric chloride solution (8 ml.). The column was eluted with water-saturated chloroform (about 50 ml.) to remove aspirin and the purple complex eluted into a volumetric flask with 10% acetic acid in chloroform (10 ml.) followed by 1% acetic acid in water-saturated chloroform to remove the complex (10). The concentration of salicylic acid was determined by measuring the absorbance of the solution at 310 mμ.

Suspension Studies—Quantities of the powder mixes to which the 2% water had not been added were placed in 30-ml. vials. Varying amounts of water from 10 ml. down to 0.1 ml. were added exactly and a few glass beads introduced to promote mixing. The vials were sealed by rubber closures and aluminum crimp caps and placed on a rotating rack (6 r.p.m.) in a water bath at 40° (±0.2°). Samples for the free salicylic acid assays were taken in the following manner: water was added to the powder mix-water suspensions in the vials to give a total volume of water of 10 ml. The rubber closure was replaced and the vial vigorously shaken for 30 sec. to dissolve salicylic acid. A 3-ml. sample was removed through a pipet with a filter on its end (glass wool in rubber tubing) and this added to 3 ml. of 2% ferric chloride solution in a volumetric flask. The flask was filled with distilled water and the solution read against a blank of the same volume without the 3-ml. sample solution at 540 mμ (3).

RESULTS AND DISCUSSION

The acceleration of aspirin hydrolysis by various common additives in both powders and tablets compressed from these powders was observed and the results are listed in Table I after allowing both powders and tablets to stand at 40° (±0.25°) for 45 days. The initial powder mix (Mix *F*) had 2% water added so aspirin degradation might proceed at a reasonably rapid and easily measurable rate. An attempt was made to use a standard type direct compression formulation although difficulties were encountered with flow of the two stearate salts and consequently their concentration was reduced to 5%. This had little bearing on the course of the study as goals were for relative ranking of various mixes rather than for any quantitative results at this time.

It is readily apparent from Table I that the tablets and powders fall into the same order regarding salicylic acid formation and thus the relative ranking numbers (between 1 and 100) show considerable similarity. The same behavior was demonstrated by samples stored at room temperature with the degree of degradation being less pronounced. This seems to indicate that the force of tablet compression has negligible effect on aspirin stability of the mixtures under scrutiny.

Table II gives the apparent zero-order rates with regard to salicylic acid formation in milligrams per hour for a saturated 1-ml. sample of the mixes. The relative ranking is seen to be the same as Table I.

Figure 1 illustrates a typical plot of salicylic acid generated *versus* time for a suspension of Mix *A* containing 594 mg. powder in 6 ml. water. The apparent zero-order rate constant can be obtained as usual from the slope of the straight line. Experiments of this sort (Fig. 1) were performed where the amount of water present in suspension was reduced to approach that of the solid dosage forms. Composites of these rate constants *versus* volumes of water in milliliters are shown in Figs. 2 and 3 for Mixes *A* and *C*, respectively. The tendency toward the anticipated zero rate of reaction at zero water concentration is obvious. Figure 4 gives the plots of suspensions with low water contents (0.1, 0.2, and 0.5 ml.) of the fast-reacting magnesium trisilicate system (*D*) in the usual units of milligrams salicylic acid (amount) *versus* time. The slope ratios deviate from the 1:2:5 which is expected from a process following the zero-order rate law. The low value obtained from the apparent zero-order rate constant in Figure 4 for the higher amount of water (0.5 ml.) is probably a result of unsaturation of the system with regard to all components. If such a system were saturated a graph of rates *versus* water volume would be linear as is the case in Figs. 2 and 3.

[2] Celite 545, Johns-Manville, New York, N. Y.
[3] When the samples showed considerable degradation a proportionally greater amount of Celite and ferric chloride solution had to be used.

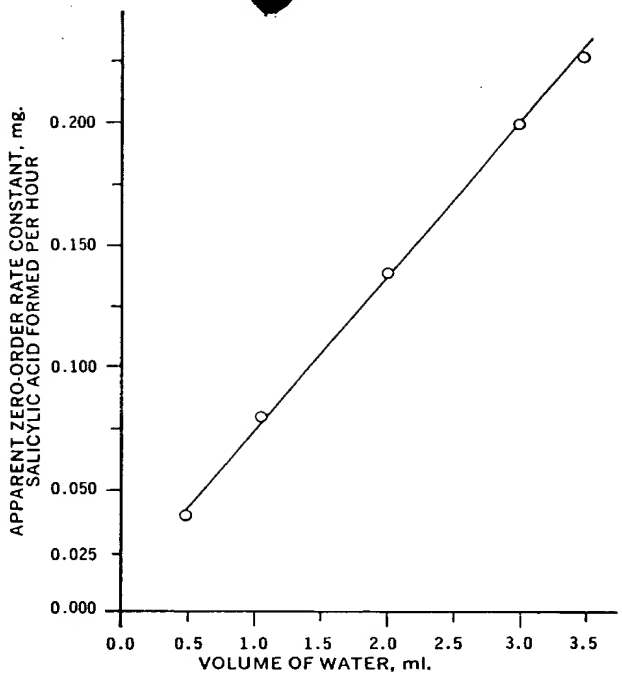

Figure 3—*Relationship of rate of salicylic acid generation to the volume of water contained in Mix* C *suspension. 10% hexamic acid is the additive. Temperature, 40°.*

The comparatively high reaction velocities encountered in systems as *D* as well as *B* and *E* (Tables I and II) may be partially accounted for by pH phenomena manifest in the suspension and in moisture present in solid dosage forms as previously mentioned by Zoglio and

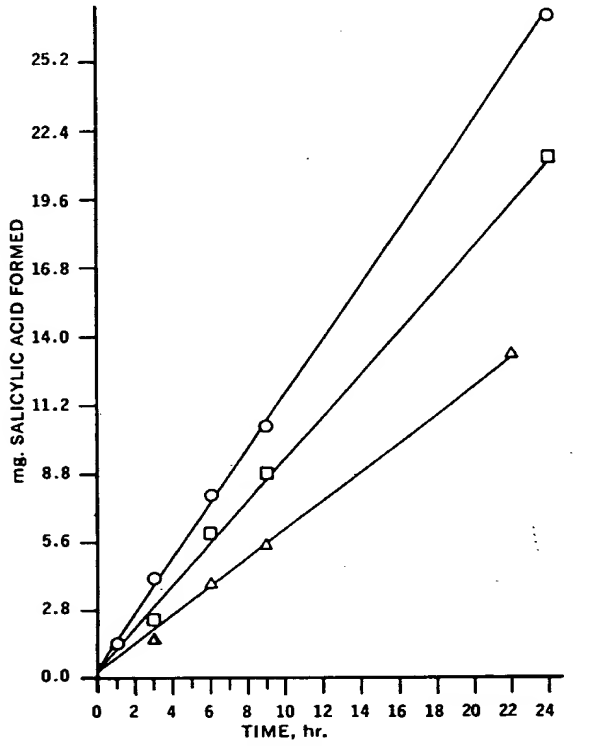

Figure 4—*Apparent zero-order plots for aspirin hydrolysis in aqueous suspensions containing 594 mg. Mix* D *(samples equivalent to 400 mg. aspirin). 10% magnesium trisilicate is the additive. Key:* ○, *slope 1.16, 0.5 ml. H_2O;* □, *slope 0.93, 0.2 ml. H_2O;* △, *slope 0.57, 0.1 ml. H_2O.*

Kornblum (3). The two mixes containing magnesium trisilicate and magnesium stearate result in generation of the freely soluble magnesium salt of aspirin producing a buffer with aspirin (3) of relatively high pH—3.5 and above—which is a poor environment for aspirin. When 1.188 g. Mix *D* was stirred in suspension with 20 ml. water the pH increased from 3.5 to 3.85 in 6 hr. while Mix *E* remained constant at 3.45 for the same time period. Over this interval of time the magnesium titer of *D* goes up to three times its initial value while *E* remains approximately at its original figure. Almost the same values hold for simple aspirin–magnesium stearate and aspirin–magnesium trisilicate suspensions. This helps explain the increased pH as it seems to be a partial result of a large amount of magnesium aspirin in solution. The pH profile of acetylsalicylic acid shows there is a considerable difference in the rate constants for degradation at these two values (3.85 for *D* and 3.45 for *E*) with the ratios being 2:1 at 25° (11). The high water content of magnesium trisilicate USP probably contributes to the degree and rate of Mix *D* relative to the others. It should be pointed out that in systems like these it is extremely unlikely that only one variable is operative but that several factors are working concomitantly to lead to the observed results. The 5% calcium stearate preparation (Mix *B*) exhibits somewhat less aspirin decomposition than 5% magnesium stearate which has previously been shown to be the case with simpler mixtures (3). The 10% aluminum hydroxide mixture (*A*) shows relatively little aspirin breakdown possibly due to the comparative insolubility of the aluminum salt of aspirin thus lowering the pH close to the saturation pH of aspirin (about 2.6) which is a fairly stable range for aspirin (11). Aluminum hydroxide is also well known as an adsorbent which may play some role in preventing aspirin decomposition.

The basic mix (*F*) and the 10% hexamic acid (*C*) show relatively little degradation as might be expected from pH effects (5).

In Table I the milligrams of salicylic acid formed in the tablets and powder mixes are expressed as the amount formed per 1% moisture. The adjustment was made when a variation of water content was found by Karl Fischer analysis (1.0 to 1.8%) rather than the expected 2%. Corrections were based on the assumption that for these systems apparent zero-order rates and therefore amounts of salicylic acid formed were directly proportional to moisture content. The adjustment to equal moisture levels in this manner provided a more successful degree of ranking.

Implicit in work of this nature is the ultimate aim of stability prediction for solid dosage forms from apparent zero-order rates obtained from suspension studies along with other pertinent information concerning the system under investigation. Long-term predictions were not attempted in this study but it is shown that relative results comparing powder mixes, tablets and suspensions exhibit very good correlation in the systems under observation as listed in Tables I and II.

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Microculture Assay for the Rapid Determination of Antifungal Activity

M. B. MRTEK, L. J. LEBEAU, F. P. SIEGEL, and R. G. MRTEK

Abstract □ Determination of hyphal growth rates in microslide cultures has been utilized to determine concentrations of griseofulvin. At concentrations between 0.001 mcg. and 0.01 mcg./ml. griseofulvin in Sabouraud liquid medium, the curve showing growth rate as a function of log dose is linear. In this concentration range the rate of hyphal elongation is constant with respect to time, and curling or distortion of hyphae is not seen except at the upper limit of the range. Microculture assays are compared to simultaneous assays performed using the current USP agar cup procedure. The microslide technique requires a combined incubation and reading period of 24 hr. It can be used to determine accurately one thousandth the concentration of griseofulvin required for the official procedure.

Keyphrases □ Antifungal activity, determination—microculture slide technique □ Griseofulvin activity, assay—agar cup *versus* microculture technique □ Hyphal growth rates—griseofulvin concentration

Although chemical assays are available for most antifungal agents, microbiological assays are the accepted standards for potency determinations (1). Microbiological assays use minute quantities of drugs with solvent extraction being the only usual preparative step required for dosage forms or biological specimens. While compounds possessing analogous chemical structure frequently interfere with chemical assay methods, this is not a problem with microbiological methods, unless the analogs also possess biological activity. Microbiological assay techniques have been extended to the determination of compounds possessing antifungal activity. Turbidimetric and agar diffusion methods have been most widely accepted as antifungal assays (1). Although several methods have been developed to observe the growth of fungi microscopically, few have been developed sufficiently to be useful as assay procedures. The objective of this investigation is to develop the microculture slide technique of Elliott *et al.* (2) as a rapid, sensitive, and efficient assay procedure for antifungal agents. Because of its potency

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REVIEW ARTICLE

Determination of the Decomposition of Aspirin

CLARK A. KELLY

Keyphrases ☐ Aspirin decomposition—determination ☐ Decomposition products—aspirin ☐ Hydrolysis, aspirin—mechanism, kinetics, pH effect ☐ Analytical methods—salicylic acid in aspirin and aspirin products ☐ Stability—aspirin and dosage forms

CONTENTS

February 27, 1917, the day U. S. Patent No. 644,077 expired, a whole new vista opened to the drug industry. What was, and is, the best seller of all times could easily be classed as the wonder drug of all ages. From the latest available figures of the U. S. Tariff Commission,[1] there would be over 200 tablets available for every man, woman, and child in the United States if all the U. S. yearly production was made into tablets containing 325 mg. (5 gr.) of acetylsalicylic acid, more popularly known as aspirin.

Aspirin has the unique standing in the medical world of still being the most widely used drug, even with the advent of modern, highly potent therapeutic agents. Aspirin has superior qualities as an antipyretic and as a general analgesic, but more specifically in the relief of headaches, muscular pain, postoperative and traumatic pain, postpartum pain, dysmenorrhea, malignancy, colds and respiratory diseases, rheumatoid arthritis, acute rheumatic fever, and in the field of dental analgesia.

With such a versatile drug, it was obvious that aspirin would be combined with other drugs with the result that a more potent and effective preparation would result. It is the purpose of this review to emphasize the plight that continually plagues the analytical chemist in his constant search for a truly reliable method of measuring the decomposition of aspirin in the presence of other drugs or compounds. What makes this problem even more acute is that aspirin is highly selective of the type of compounds it intimately associates with. In fact, if aspirin acquires even a trace of moisture, it begins to fall to pieces. It thus becomes a problem of product development to finalize a stable formulation that will withstand a "normal" shelflife under all types of adverse conditions such as humidity, temperature, and interreactions with other components, even in the solid state.

DETERMINATION OF DECOMPOSITION OF ASPIRIN

When aspirin was first introduced as a drug, controversies ensued almost instantaneously as to how one could characterize truly good aspirin. Some became experts on detecting trace amounts of acetic acid and so classed the elegance of the aspirin accordingly. Even in those early days, the advertising agencies made the most

[1] From 1968 preliminary report: 31,248,000 pounds of aspirin by U. S. production.

of this purely subjective classification. Very few papers have been presented on the quantitative determination of acetic acid as a decomposition product of aspirin, chiefly because of the known volatility of acetic acid. Unless the original container was completely airtight, one would be measuring only the residual acetic acid, which would not be representative of the total acetic acid formed by the decomposition of the aspirin. General methods evolved in which dry air was passed over and through a thin layer of the finely powdered sample. The acetic acid vapor was trapped in the water and then titrated with very dilute sodium hydroxide. A simpler approach utilized a Conway micro diffusion cell. The more refined approach involved GLC.

It is interesting to note that visually the presence of any whiskers (very thin elongated crystals of salicylic acid) observed on the surface of a solid product containing aspirin is definitely an indication that some of the aspirin has decomposed and that the resulting salicylic acid has sublimed through the solid material. Here, again, if the container is not airtight, the possibility exists whereby the released salicylic acid, through sublimation, would leave the sample area and so not be measured. This crucial point, on the sublimation of salicylic acid, will be discussed more thoroughly in this review. As with most subjective tests, the evaluation of solid aspirin products by the appearance of salicylic acid whiskers is limited. Time is required for this sublimation to take place, so one would not normally apply it to fresh products. Therefore, one could actually have a poorly made aspirin tablet which, on the surface, showed no whiskers but internally had a high content of salicylic acid.

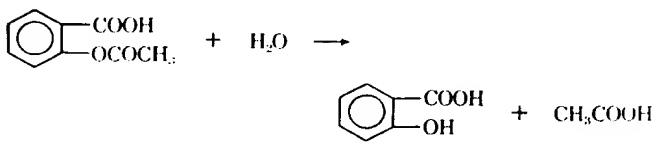
It is the intent of this review, therefore, to pursue a quantitative approach in the determination of the amount of decomposition of aspirin through the presence of salicylic acid rather than acetic acid.

No biological samples, such as blood, containing aspirin will be discussed in this review. Nor will this review include salts of aspirin, such as aluminum aspirin, solutions, or aspirin suspensions. The stability of aspirin in all these cases is definitely limited. The presence of any moisture (with the salts of aspirin, the water of hydration) results in the hydrolysis of aspirin at such a rate that the given formulation does not have a practical or useful shelflife. There is, however, a definite need in the field of pediatrics and geriatrics for a stable liquid formulation of aspirin, because there is no easier or simplier way to give a medicinal than by mouth to infants or to the feeble.

Even limiting this review just to solid preparations leaves a great deal confronting the analyst. A step-by-step evaluation of the approaches published in the scientific literature will be presented. One will readily see the tremendous need for a simple, reliable, universal test for the decomposition of aspirin which can be applied easily and rapidly to a completely unknown preparation containing aspirin.

HYDROLYSIS STUDIES

Decomposition of aspirin results from hydrolysis of the ester group, with the end products being acetic acid and salicylic acid. The oversimplified reaction for the hydrolysis of aspirin is presented only at this time, so one may visualize the overall picture of the decomposition of aspirin (Scheme I).



Scheme I

Those who have made a thorough study of the hydrolysis of aspirin under various well-controlled and stated conditions report that the reaction is very complex. Judged by the number of papers on this subject alone, the reaction is also highly controversial.

The first publication on the hydrolysis of aspirin in water was reported by Rath (1) who conducted his work at an extremely high temperature (100°). As was later found, the hydrolysis of aspirin is very sensitive to temperature changes, even near room temperature. The rate of hydrolysis was determined by titrating the total acidity at stated time intervals. The calculated values indicated a monomolecular reaction. Tsakalotos and Horsch (2, 3) also followed the hydrolysis of aspirin but at more reasonable temperatures (20, 50, and 60°). It took about 100 days to effect complete hydrolysis of aspirin in water at room temperature. Hydrogen ion was found to accelerate the hydrolysis rate, hydrochloric acid being more effective than sulfuric acid. Acetic acid and citric acid caused an initial increase in the rate of hydrolysis; but as the days passed, a decrease in the rate of hydrolysis was noted. The unfounded explanation given by these authors for this slowdown was that the salicylic acid produced was being acylated.

Wolf (4) substantiated the hydrogen-ion effect on the hydrolysis of aspirin by showing that the velocity constant in an acid medium (diluted hydrochloric acid) doubled over that of just water

Aspirin was solubilized in water at room temperature by Morton (5) with the aid of potassium and sodium citrates and acetates. The degree of hydrolysis was followed by titrating the samples with standard alkali at stated time intervals. The rate of hydrolysis was reported to be independent of not only the concentration of the aspirin but also of the solubilizing salt concentration.

Saponification (alkaline hydrolysis) of aspirin was reported by La Mer and Greenspan (6) at 25.000 ± 0.005°. The reaction was stopped by making the given sample (not an aliquot of the bulk solution as is the usual approach in hydrolysis studies) acidic with standard sulfuric acid. The excess acid was then titrated with 0.02 *M* sodium hydroxide. It is assumed that this back titration was conducted immediately; otherwise, hydrolysis of aspirin in the strongly acidic medium would become an unwanted factor in the calculation of the saponification rate. This study showed that aspirin underwent a simple ionic bimolecular reaction with sodium hydroxide in aqueous solution. Thus, a second-order rate constant was calculated.

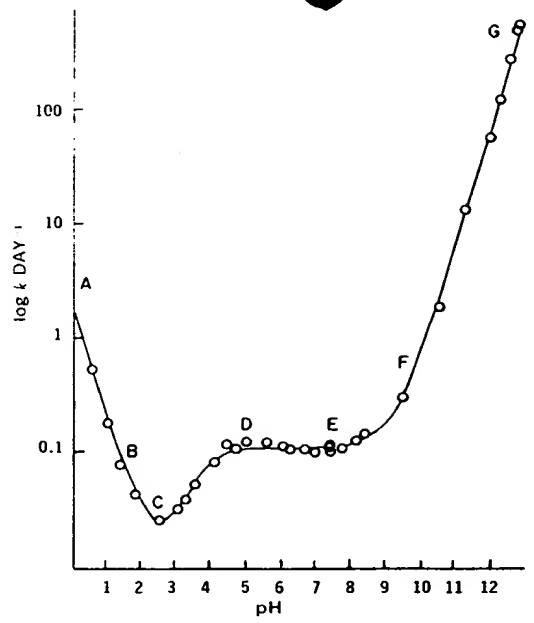


Figure 1—*pH–rate profile for hydrolysis of aspirin. [Reprinted, with permission, from L. J. Edwards,* Trans. Faraday Soc., **46**, *723(1950).]*

Continuing this same approach, Sturtevant (7) analyzed the rate of saponification of aspirin at 35° calorimetrically with the aid of a thermocouple. His conclusion is not often seen in print other than by authors commenting about the previous contributions in relationship to their own work: "The results of these experiments have shown, however, that it would be very difficult to get accurate heat data on these reactions, and it has, therefore, not been considered worthwhile to carry the measurements any further."

A complete and thorough kinetic study of the factors involved in the hydrolysis of aspirin in dilute solution (3×10^{-3} *M*) was conducted by Edwards (8, 9). Using a UV spectrophotometric method for simultaneous determination of aspirin and salicylic acid, he observed the rate of decomposition to be first order at a fixed pH value (between pH 0.53 and 12.77) and constant ionic strength at 17°. Figure 1 depicts the relationship between velocity (rate) constant and pH. This curve was subject to only slight alteration with change in ionic strength. Temperature dependence of this aspirin reaction was studied between 10 and 50°. The pH–rate profile was of the same shape for every temperature, with displacement upward with increasing temperature. This plot of log *k* against pH helps to show visually that the hydrolysis was catalyzed appreciably by hydrogen ion (section AB of figure) and very strongly by hydroxyl ion (section FG of figure). Over the pH range 5–8 (section DE of figure), the rate was constant; in the pH range 2–3 (section C of figure), there was a pronounced minimum rate where the reaction velocity dropped to less than a quarter of the stationary value (DE) which is usually taken to represent the "spontaneous reaction." Edwards explained the relationship between the rate constant and pH on the assumption that the hydrolysis of aspirin may take place *via* the six simultaneous reactions shown in Scheme II.

Table I—Comparison of Hydrolysis Rates of Aspirin in Various Media

Investigator	*k*, Day^{-1}	Medium
Rath (1)	4.35×10^{-2}	Water
Edwards (8)	4.1×10^{-2}	Water
La Mer and Greenspan (6)	7.05×10^{3}	Sodium hydroxide
Sturtevant (7)	7.2×10^{2}	Sodium hydroxide
Edwards (8)	7.50×10^{3}	Sodium hydroxide
Morton (5)	0.103	Potassium citrate buffer about pH 7
Edwards (8)	0.117	pH 7

Through many relationships involving these six equations, the observed unimolecular (first-order) velocity constant could be expressed as a function of the six second-order constants (Eq. 1):

$$k = \frac{k_1 C_H + k_2 C_{H_2O} + k_3 C_{OH}}{1 + K/C_H} + \frac{k_4 C_H + k_5 C_{H_2O} + k_6 C_{OH}}{1 + C_H/K} \qquad \text{(Eq. 1)}$$

When each of the six components of *k* was plotted on a pH–log *k* diagram, four types of curves were obtained. The combination of these four individual curves into one overall curve resulted in a single final curve similar to that depicted in Fig. 1, including the previously inexplicable minimum (C in figure), which Edwards calculated as being at pH 2.44. (The observed minimum was at pH 2.5.)

A comparison of the results of this work (Table I) with those obtained by previous investigators showed good agreement in the rate constant when expressed in the same terminology and conditions.

The hydrolysis of aspirin was accounted for over the whole pH range by considering all the possible bimolecular reactions between the five species present in the equilibrium as: $2H_2O \rightleftarrows H_3O^+ + OH^-$ on the one hand and the equilibrium: $CH_3COOC_6H_4COOH \rightleftarrows CH_3COOC_6H_4COO^- + H^+$ on the other hand.

The mechanism of intramolecular catalysis of the hy-

	k, Day^{-1}
$CH_3COOC_6H_4COOH + H_2O^+ \xrightarrow{k_1} HOC_6H_4COOH + CH_3COOH + H^+$	1.9
$CH_3COOC_6H_4COOH + H_2O \xrightarrow{k_2} HOC_6H_4COOH + CH_3COOH$	$\gg 10^{-6}$
$CH_3COOC_6H_4COOH + OH^- \xrightarrow{k_3} HOC_6H_4COOH + CH_3COO^-$ *or* $HOC_6H_4COO^- + CH_3COOH$	$\ll 10^{6}$ and probably $< 10^{3}$
$CH_3COOC_6H_4COO^- + H_3O^+ \xrightarrow{k_4} HOC_6H_4COOH + CH_3COOH$	46.7
$CH_3COOC_6H_4COO^- + H_2O \xrightarrow{k_5} HOC_6H_4COOH + CH_3COO^-$ *or* $HOC_6H_4COO^- + CH_3COOH$	2.11×10^{-3}
$CH_3COOC_6H_4COO^- + OH^- \xrightarrow{k_6} HOC_6H_4COO^- + CH_3COO^-$	7.5×10^{2}

Scheme II

drolysis of aspirin in the pH region of 5–8 led Davidson and Auerbach (10) to investigate the behavior of aspirin in nonaqueous media. In the presence of dissolved base, aspirin possessed acid anhydride properties and functioned as an effective acetylating agent. They postulated the existence of a cyclic intermediate which results from the intramolecular nucleophilic attack by the ionized carboxyl group on the ester carbonyl. They also postulated that this base-catalyzed isomerization was the rate-controlling step for the hydrolysis of aspirin in the pH 5–8 range. This work was done with organic solvents, and there was no evidence for reactions of this type in aqueous solution.

Ferroni and Baistrocchi (11) measured the rate of hydrolysis of aspirin by determining the liberated salicylic acid fluorometrically. They reported that the reaction followed first-order kinetics and the rate constant was evaluated as 0.0122 $hr.^{-1}$ at 18.5° and pH 9.42.

Hydrolysis rate constants for aspirin, reported by Miyamoto *et al.* (12), agreed with the k_5 rate constant of Edwards (8): 2.625×10^{-3} at 24° and 6.909×10^{-3} at 37°. A general comment was made that aspirin hydrolyzed more quickly in simulated intestinal fluid than in simulated gastric fluid.

Garrett (13), extending the work of Edwards (8) to include a number of acyl salicylates in a very complete investigation, looked more thoroughly into the pH–rate profile of aspirin hydrolysis, particularly in the pH 4–8 range. Edwards' own demonstration that the hydrolysis was not catalyzed by acetate ion (varied from 0.005 to 0.3 *M*) was not consistent with the mechanism involving an attack by a water molecule on the aspirin anion, because the acetate ion is a considerably more powerful nucleophile than water. Garrett's work pointed rather to intramolecular nucleophilic catalysis by the ionized carboxyl group. Even though the carboxylate ion is an unfavorable case from the point of view of nucleophilicity, it apparently participates catalytically in a number of intramolecular catalyses of esters. The hydrolysis of aspirin may be regarded as a classical example. On increasing the alcohol concentration greatly in the pH-independent region of the pH–rate profile, a very unexpected increase was found.

In this light, alcohol would have to be considered a more active nucleophile than water. To clarify this anomalous enhancement of "spontaneous" hydrolysis with increasing alcohol content (0–60%), Garrett made a thorough study. The addition of alcohol to the solvent increased the rate of solvolysis; ethyl acetate was a resulting product. He ruled out the possibility that the rate increase was a generalized solvent effect by showing that the addition of dioxane had very little effect on the rate of hydrolysis of aspirin. He tried to explain his results by proposing a mechanism involving nucleophilic attack by alcohol on the tetrahedral carbon atom of an intermediate compound. This explanation has not been generally accepted. Nevertheless, the demonstration that the addition of alcohol increased the rate of solvolysis did suggest strongly that the question of the involvement of a molecule of solvent in the transition stage ought to be studied.

Using the same four acyl esters of salicylic acid as in the mentioned studies, Garrett (14) studied the stability of their saturated solutions. Prediction of stability in such solutions was made from separate studies of solubility rates and homogeneous rates on dilute solutions of these esters, since solvolytic degradation was a function of these two rates. This study did show that aspirin was the least stable of the esters studied.

Okano and Kojima (15) investigated the effect of salicylic acid upon the rate of aspirin decomposition in solution. They observed deceleration of the aspirin hydrolysis between pH 2.2 and 7 with increasing amounts of salicylic acid being added to the medium. Below pH 2.2 the opposite effect was noted. These studies were conducted for 10 days at 35°, or 8 hr. at 50°, and the rate constants were calculated. The reversal effect of the salicylic acid on the hydrolysis of the aspirin is in the vicinity of the minimum shown by Edwards' (8) pH–rate profile of aspirin hydrolysis.

The hydrolysis of aspirin at pH 6 in water containing 4.3 atom % of ^{18}O produced, after 22 hr. refluxing, salicylic acid containing 6% of the excess ^{18}O in the water. This result was in agreement with the theoretical prediction made by Bender *et al.* (16) and gave backing to the hydrolysis mechanism of aspirin postulated by Garrett (14) and others. This involved an intramolecular attack of the carboxylate ion on the carbonyl carbon atom of the ester to produce acetylsalicyl anhydride, which subsequently hydrolyzes rapidly to produce acetate and salicylate ion, or alternatively that the addition of the carboxylate ion to the carbonyl group of the ester is followed by some reaction with water leading to the same products. It can be calculated from the relative rates of hydrolysis of ethyl acetate and ethyl salicylate that the reaction producing salicylic acid-^{18}O should occur to the extent of 2.5%. The 6% observed was considered to be reasonable and consistent with the postulated reaction in which water was involved.

Using a BPC mixture of aspirin, James (17) investigated the kinetics of the hydrolysis of aspirin from aqueous suspension by comparative, rather than quantitative, means. As long as there was a good excess of aspirin suspension present at the different temperatures, the hydrolysis rate was zero order. Thus, the more concentrated the suspension, the more stable was the aspirin. After 62 days at room temperature, suspensions of 3.3, 6.5, and 13.0% aspirin showed the following percent of intact aspirin remaining: 90, 94, and 97%, respectively. This follows, as the hydrolysis rate depends on the amount of aspirin in solution. Hence, suspensions show a low degree of hydrolysis relative to the total amount of aspirin in suspension.

While James used a titration procedure to follow the rate of hydrolysis in his study, Blaug and Wesolowski (18) used a more refined UV procedure in a pH 3 buffer. This pH was selected because it is the pH of a saturated solution of aspirin (approximately 4 g./l.). The effect of the following additives (calcium gluconate, glycerin, *N*-methyl-2-pyrrolidone, polyethylene glycol 6000,[2] polyvinylpyrrolidone, salicylic acid, sorbitol, pH 3.0 buffer, and water) on the stability of aspirin suspensions (6.5% of 100-mesh and 13% of 60-mesh aspirin) was followed

[2] Carbowax 6000, Union Carbide Corp.

by measuring the salicylic acid content at 298 mμ. The thermodynamic values reported in the paper were calculated from the data obtained with these various suspensions. The suspensions (in duplicate) were stored in a 50 ± 0.5° mechanical shaker for 24 hr. Samples were removed from the suspensions at hourly intervals for assay. Calcium gluconate accelerated the hydrolysis by increasing the pH of the medium, while the *N*-methyl-2-pyrrolidone or glycerin enhanced the solubility of the aspirin, thus increasing the hydrolysis. The presence of saturated salicylic acid did not affect the hydrolysis rate. The most promising additives were polyethylene glycol 6000 and polyvinylpyrrolidone, but physically they were unsatisfactory at this temperature as they formed gummy, insoluble masses of the suspension. Only sorbitol showed any potential stabilizing effect on the aspirin suspension.

In continuing his studies on the effect of alcohol on the hydrolysis rate of aspirin, Garrett (19) synthesized the mixed anhydride of aspirin and acetic acid to help establish his mechanism for the hydrolysis reaction. This compound did not form ethyl acetate as expected in the alcohol medium, so a logical explanation regarding the experimental evidence was still lacking.

Further studies with deuterium oxide solvent isotope effects in the nucleophilic reactions of phenylesters were reported by Bender *et al.* (20). One of the continuing problems associated with the hydrolytic reactions of carboxylic acid derivatives is to distinguish between nucleophilic and general basic catalysis of hydrolysis. The former involves the attack of a nucleophile upon a substrate, leading to the formation of an unstable intermediate which spontaneously breaks down to give the product and regenerates the catalytic entity. The latter catalysis involves the attack of a general base on the substrate removing a proton in a rate-determining stage. Either of these two processes may be carried out by a given substance which, by definition, is at one and the same time both a nucleophile and a general base. The deuterium oxide solvent isotope effect has been used to distinguish between these two possibilities.

The aspirin hydrolysis has been shown, on the basis of kinetic and isotopic experiments, to involve an intramolecular nucleophilic-catalyzed hydrolysis involving an anhydride intermediate. For the purpose of calculation, it has been assumed that the transition state of the reaction was one in which the carboxylate ion has been added to the carbonyl group of the ester, forming a tetrahedral addition intermediate. The formation of this intermediate is, in general, the slow step in the nucleophilic reactions of carboxylic acid derivatives. The authors concluded that the use of deuterium oxide solvent isotope effects as a criterion to distinguish between general base- and nucleophilic-catalyzed reactions was ambiguous; but when applied in a restricted sense, it may be empirically rewarding.

Nogami *et al.* (21) examined the effect of cationic (cetylethyldimethyl ammonium bromide and benzalkonium chloride), anionic (sodium lauryl sulfate), and nonionic (polyoxyethylene lauryl ether) surfactants on the suppression of the hydrolysis of aspirin which exists in anionic and undissociated forms in aqueous solution. The decomposition-rate constants in the buffer solutions (pH 1–7.5) were obtained, with or without the surfactant, and compared. Samples were kept at 37 ± 0.1°, with aliquots being removed at given intervals and assayed for salicylic acid with a ferric nitrate reagent. The color was determined spectrophotometrically at 530 mμ.

The hydrolysis of aspirin was found to follow a pseudo-first-order reaction in the media studied. In the pH 5–7.5 range, aspirin was chiefly in the anionic form. Due to electrostatic attraction, it formed a complex with the cationic surfactant which moved into micelles composed of excess surfactant. Thus, the hydrolysis of aspirin in this pH region was suppressed only by a cationic surfactant. In the pH 1–5 range, all the surfactants suppressed the hydrolysis of aspirin. Because the undissociated aspirin existed in this region, it moved into micelles and was less hydrolyzable. Near pH 1, only the anionic surfactant lost its effect on suppressing the hydrolysis of aspirin. This was explained by the promoting effect of sodium lauryl sulfate on the hydrolysis of aspirin, due to the attracted hydrogen ion on the micelle environment competing with the suppressing effect of the solubilization. Even though this report shows suppression of the hydrolysis of unionized aspirin by all the surfactants and the suppression of the anionic form by cationic surfactants, it by no means implies that these solutions could be used as a stable pharmaceutical formulation.

Nelander (22) reported the heat of hydrolysis of aspirin at 25° by a calorimetric procedure. The $-\Delta H$ (kcal./mole) for aspirin was 25.39 ± 0.03 in 0.8 *N* sodium hydroxide in water–alcohol, 2:3. The heat of solution in aqueous tromethamine solution, ionic strength 0.1, initial pH 8.05, for aspirin was 5.72 ± 0.08 kcal./mole.

The first application of ultrasonic energy in accelerated drug stability studies was published by Mario and Gerraughty (23). Duplicate samples of aspirin in the given buffer (pH 2.00, 4.00, or 5.95) were put in two constant-temperature baths at 21, 25, 35, and 45°, one with ultrasonic energy and the other (control) without. Aliquots from both baths were taken at stated time intervals and assayed for salicylic acid at 302 mμ; the content was calculated from standard curves of salicylic acid in the same buffer. Aspirin runs were done at two different concentration levels. The agreement of duplicate runs was good and indicated that the experimental technique was reproducible.

Pseudo-first-order rates were found at all pH values and with varying temperatures, both with and without ultrasonic energy. The hydrolysis rate constant, *k*, was calculated. The Arrhenius relationship was followed in all cases, and the heat of activation of the hydrolytic degradation of aspirin was not changed by the introduction of the ultrasonic energy. The increase in the rate found with the ultrasonic samples was equivalent to increasing the reaction mixture temperature within the range of 1.8–2.9°. This range was consistent, regardless of the pH or temperature used. Although the effects of ultrasonic energy were not startling on increasing hydrolysis, these studies did show the potential of this new technique, particularly with heat labile ingredients.

Table II—Apparent Zero-Order Rate Constants of Salicylic Acid at Constant pH

Aspirin plus Lubricant	k_0, mg. FSA/hr.	pH
None	0.123	2.60
Stearic acid	0.133	2.62
Hydrogenated vegetable oil	0.123	2.68
Talc	0.133	2.71
Aluminum stearate	0.281	3.16
Calcium stearate	0.986	3.75
Magnesium stearate	1.314	4.14

Needham and Gerraughty (24) pursued further the hydrolysis of aspirin in mixed solvent systems by ultrasonic energy. The solvent systems were: alcohol–water, 10, 30, 50, and 70%; ether–water, 3 and 5%; and ethylene glycol–water, 5, 10, 30, and 50%. The pH for all of the media was kept at 3.67. Since the thermal energy (use of dual constant-temperature baths) was kept constant for both ultrasonified and control systems, it was apparent that the ultrasonic energy was responsible for the increase in the kinetic rates. With the ethylene glycol–water system, as the concentration ratio was increased, the subsequent increase in viscosity apparently reduced the movement of molecules caused by the ultrasonic vibration, as shown by the smaller rate constants for the hydrolysis of aspirin.

In studying the interaction of aspirin with urea, Santopadre and Bolton (25) shook saturated solutions of aspirin in water at 30° with known varying amounts of urea (0 through 10 *M*) for 5 hr. Kinetic studies were made at pH 2.0, 2.5, 2.75, 3.0, and 3.5 at 30 ± 0.2°. First-order rate constants were calculated. Urea increased the rate of hydrolysis below pH 2.75 and decreased the rate of hydrolysis at pH values greater than 2.75. It is interesting to note that this "crossover" occurs at a pH corresponding to the pH of maximum stability, as reported by Edwards (8). This pH may thus represent a point where the hydrolysis mechanism changes, and this could provide an explanation for the change in the effect of urea.

Murthy and Rippie (26) studied the hydrolysis of aspirin in the presence of polysorbate 80. Saturated solutions of aspirin at 30 ± 0.1° were prepared in 0, 1, 2.5, and 4% solutions of polysorbate 80 at the following pH's: 2.63, 3.63, 4.10, 4.21, and 4.43. Kinetic studies were carried out on suspensions, saturated solutions, and half-saturated solutions for 48 hr. at 30 ± 0.1°. Samples were removed at stated times and assayed by the UV method described by Edwards (8).

With the suspensions, the observed increase in degradation-rate constants (pseudo-zero-order) with added polysorbate 80 was due to the instability of undissociated aspirin in the micellar phase. With the homogeneous solutions, the rate of hydrolysis of aspirin in the polysorbate micelles, while lower than in the aqueous phase, was not negligible. The solubility determinations in the various media showed the absence of dissociated aspirin in the micellar pseudophase of the polysorbate 80 solutions.

Hydrolysis of solubilized aspirin in the presence of the nonionic surfactant, cetomacrogol, was studied by Mitchell and Broadhead (27). All the studies were conducted at 37 ± 0.1° in the pH range of 1–7 on solutions of aspirin with cetomacrogol concentrations of 0.1 through 0.07 *M*.

The hydrolysis of aspirin proceeds as a first-order reaction, both in aqueous buffer and in buffered cetomacrogol solutions. Reaction rate constants were determined. At the pH of maximum stability, pH 2.27, where aspirin exists largely in the unionized form, the half-life increased with cetomacrogol concentration. In 0.07 *M* cetomacrogol, the half-life was approximately twice that in the control buffer. In the plateau region where aspirin is largely ionized, the rate of hydrolysis was independent of cetomacrogol concentration.

Kornblum and Zoglio (28) evaluated the commonly used tablet lubricants as to their effect on the stability of aspirin. Suspensions of aspirin with the various lubricants (talc, hydrogenated vegetable oil,[3] stearic acid, aluminum stearate, calcium stearate, and magnesium stearate) were prepared. The lubricants were also in excess to ensure saturation through the experiments.

The suspensions were maintained at 30°, with appropriate aliquots withdrawn at various time intervals for pH and salicylic acid determination by adding ferric chloride and reading at 540 mμ in a spectrophotometer. From the kinetic studies of these suspensions, the reaction rate appeared to be of zero order. The pH remained relatively constant through the 30-hr. study for the given suspension.

The results are summarized in Table II.

With both calcium and magnesium stearates, the rate of decomposition of aspirin was due to more than just the increase in pH. The authors showed that this increase was due to the high solubility of calcium and magnesium aspirin which were formed in these suspensions. The mechanism primarily involves a reaction of the alkali cation with aspirin in a solution to form a salt of aspirin which, in the presence of solvated aspirin, comprises a buffer system at a pH detrimental to the stability of aspirin.

Reduction of the water content in the aspirin–calcium stearate suspension was done to approach that found in a solid dosage form, the ultimate aim being the achievement of reproducible data which would permit subsequent extrapolation to the tablet or capsule dosage form. A major conclusion from this interesting study is that stearate salts should be avoided as tablet lubricants in preparing aspirin formulations.

In their study on salicylic acid sublimation, Gore *et al.* (29) determined the hydrolysis rates for aspirin at temperatures ranging from 17.2 to 30.2 ± 0.1° at pH 7.4 and reading the resulting salicylic acid at 296.5 mμ. Their data are summarized in Table III. These values are in close agreement with those reported by Morton (5) and Edwards (8).

It is only by pure coincidence, but certainly quite appropriate, that the last papers dealing with hydrolysis of aspirin in this review clarified the situation immensely. In an attempt to circumvent the problems raised by the kinetic equivalence of the several possible mechanisms, Fersht and Kirby (30, 31) looked first at

[3] Sterotex, Capitol City Products, Columbus, Ohio.

Table III—Rate Constants for the Hydrolysis of Aspirin in pH 7.40 Buffer Solution at Various Temperatures

Temperature	k, Day^{-1}
17.2	0.0937
21.3	0.1506
25.5	0.2067
30.2	0.3429

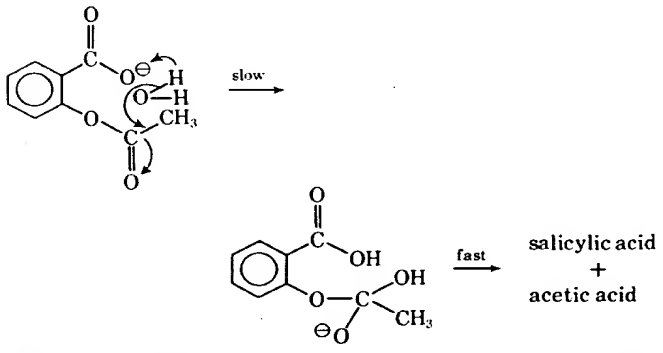
the reactivity toward hydrolysis of a series of substituted aspirins. The results suggested, unambiguously, that the most likely mechanism for the hydrolysis of aspirin was one in which the carboxylate group acts not as a nucleophile but as a general base.

The rate of hydrolysis of aspirin was measured in these studies by following the initial rate of release of salicylate at the isosbestic point, 298.5 mμ, and at 39.0 ± 0.03°. The ionic strength was maintained at 1.0 with added potassium chloride. It was this fact that showed why Edwards (8) failed to detect catalysis by acetate ion, because the ionic strength was not kept constant in his experiments. Fersht and Kirby (30, 31) found that the small acceleration due to the addition of a given concentration of acetate was almost exactly equal to the opposite effect of the increase in ionic strength. If Edwards had only known this fact, there no doubt would have been fewer controversial papers dealing with the mechanism of the hydrolysis of aspirin.

The pH–rate profile for aspirin hydrolysis, measured by Edwards (8), shows that the transition state for hydrolysis in the pH-independent region involves the aspirin anion, either alone in a unimolecular reaction or together with one or more molecules of solvent. Three mechanisms were consistent with this kinetic result for intramolecular catalysis of the hydrolysis of aspirin by the carboxyl group:

1. A unimolecular process in which the carboxylate group acts as a nucleophile. There was no longer any evidence that specifically supported the nucleophilic mechanism. It was not consistent with the effect of substituents on the reaction, and there were several indications that the rate-determining step was not a unimolecular process.

2. A general acid catalysis of the attack of hydroxide ion by the undissociated carboxylic acid group. This mechanism was rejected because intermolecular general acid catalysis by the carboxy group of aspirin should be observed for attack by acetate as well as by the hydroxide ion.



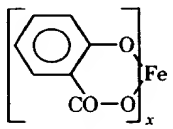
Scheme III—Mechanism of hydrolysis of aspirin as a classical general base catalysis

3. A general base catalysis of the attack of a water molecule by the carboxylate anion. There seems little doubt that the intermolecular reaction of acetate with the aspirin anion represents general base catalysis. There is even less doubt that intramolecular catalysis of hydrolysis by the carboxylate group of aspirin involves the same mechanism as the intermolecular reaction with acetate ion.

In actuality, it seems probable that the aspirin reaction lies close to the borderline between nucleophilic and general base catalysis (Scheme III).

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS WITH FERRIC IRON

Until 1965, the most prominent method of determining salicylic acid in aspirin, or products containing aspirin, was the reaction with ferric iron under a variety of conditions. The complex produced on mixing ferric ion and salicylic acid (a bidentate ligand) results in the formation of a series of intensely colored metal chelates having ligand–ferric-ion ratios of 1:1, 2:1, and 3:1. The formation of the cyclic chelate structure (I) involves the displacement of the weakly acidic, phenolic hydrogen by the metal, resulting in the formation of a six-membered ring by coordination of the metal through the phenolate and carboxylate groups of salicylic acid:



I

In the first of these publications, in 1911 (32), the sample containing aspirin was shaken with water or alcohol and filtered; then one drop of ferric chloride solution was added. On standing, the color changed from reddish to dark violet. This, in essence, was the beginning of the official compendia tests for salicylic acid in aspirin. It was noted very early by Melzer (33) that the presence of either sodium phosphate, tartaric acid, or borax masked the iron–salicylic acid color reaction. The useful suggestion was made that the tablets first be extracted with ether, since only the aspirin and salicylic acid would be extracted; thus a simple separation from the interfering compounds was easily accomplished. This immediately brings to mind that it would be easy to obtain false negative tests for salicylic acid if such a compound was incorporated in the aspirin tablet and no prior separation were made. Before the year was over, Linke (32) had refined the method for determining free salicylic acid in aspirin tablets by comparing the resulting iron–salicylate color to a series of salicylic acid standards. As little as 1 mcg. salicylic acid/ml. could be detected.

A limit test of 0.1% salicylic acid in aspirin was described by Leech (34), using a small volume of alcohol to dissolve the aspirin completely and then diluting with water before the addition of the ferric chloride reagent. This test became the basis for USP procedure when aspirin became official in 1926. It was emphasized that the standard salicylic acid tube should contain the same

amount of alcohol as the sample, because the final color was affected (decreased) by the presence of alcohol. Jones (35) stated that for tablets the limit test should be 0.15% salicylic acid and that there should be no turbidity in the final solutions.

Rather than compare the salicylic acid extract from aspirin, as described, Dahm (36) prepared a permanent color series containing various amounts of cobalt chloride dihydrate. After standardization with known quantities of salicylic acid, one could compare directly, under the stated conditions, aspirin extracts and "read" the percent free salicylic acid (FSA) directly. As these cobalt colors were of a permanent nature, it would save the analyst preparing a fresh salicylic acid reference standard.

Comments about the BP salicylic acid test made by Nutter-Smith (37, 38) brought forth that the official test was not effective below 0.04% salicylic acid, because the ferric chloride reagent was the limiting factor due to its own color. Using ferric ammonium sulfate corrected this situation. The presence of tartaric or citric acid in tablets (1%) has been shown to mask the presence of 0.2% salicylic acid. Thus, if one obtains a negative test for the FSA in unknown aspirin tablets, proper steps should be taken to separate the salicylic acid from the interfering material. Ruddiman (39, 40) added oxalic and tannic acids as masking agents of the iron–salicylate test and commented that sodium phosphate and borax did not interfere with the iron–salicylate test as had been believed. Incompatibility of aspirin with many drugs was proven by Snidow and Langenhan (41) using ferric alum reagent qualitatively.

Valentin and Lieber (42) showed that if ether was used to extract aspirin and salicylic acid from other materials, the evaporation step must be done with care, because too much heat results in high FSA values. They suggested chilling the ether and passing a stream of air over the solution to expedite the evaporation.

The use of a spectrophotometer in the determination of FSA was introduced by Hoffman (43) in 1929. Since the tablets being analyzed contained magnesium oxide, 4 *N* sulfuric acid was added during the grinding of the tablets. This was added to prevent hydrolysis (although not stated, it would also free any salicylic acid which might have been combined with magnesium ion) of the aspirin before the salicylic acid was extracted with a 1:1 mixture of ether and pentane. After evaporation of the clear extract, alcohol was added to dissolve the salicylic acid, and the iron reagent (ferric chloride) was added. This color was then compared with a standard series in the spectrophotometer.

Chloroform was introduced as a direct extractant of aspirin by Hitchens (44). This chloroform extract was shaken with 2% sodium bicarbonate aqueous solution to remove the aspirin (and salicylic acid). This, in turn, was made acidic with hydrochloric acid and extracted quantitatively with ethyl acetate. The ethyl acetate extract was evaporated under reduced pressure in a water bath maintained between 40–45°. The residue was dissolved in alcohol and diluted with water; ferric ammonium sulfate solution was then added. The resulting color was compared with standards of salicylic acid in the same medium. Because hydrolysis does take place in alkaline medium, Hitchens showed that, at 20° for 1 hr. in the sodium bicarbonate solution, about 0.25% of the aspirin was hydrolyzed. At 30°, about 0.35% of the aspirin was hydrolyzed. Since the procedure described took less than 20 min. in the alkaline medium, the error caused by this alkaline hydrolysis was called negligible. With standard runs of aspirin USP, the FSA content was less than 0.15% by this extraction procedure. With various mixes and commerical tablets, the FSA found was never over 0.2%. The purpose of this extraction procedure was to isolate quantitatively the aspirin from compounds such as acetphenetidin, caffeine, acetanilid, antipyrine, amidopyrine, and phenylsalicylate.

In 1937, Banchetti (45) made a critical evaluation of many of the pharmacopeias in regard to their FSA tests which used various ferric iron reagents after extraction of the aspirin and salicylic acid. It was pointed out that the tests should be done at the lowest practical temperature and as rapidly as possible to minimize hydrolysis of the aspirin while conducting the procedure. If evaporation of a solvent extract is required, it should be done with as little heat as possible to avoid excessive FSA values.

The first reported humidity- and temperature-controlled experiments with aspirin tablets in different packagings was conducted by Canback (46). Tablets were stored in wood boxes, tins, impregnated paper, and glass bottles at 20 ± 0.2° for 1 year at various humidity stations (0, 19, 44, 59, 75, and 100% relative humidity). Using 2.5 *M* acid to acidify the pulverized powder, the aspirin and salicylic acid were extracted with a 1:1 mixture of ether and petroleum ether. An aliquot was evaporated, and the salicylic content was determined by dissolving the residue with diluted alcohol. Ferric chloride solution was added, and the resulting color was read in a colorimeter after standing 15 min. After a year at the various humidity stations, the aspirin tablets stored in glass showed very little change in FSA content. The other packagings were greatly inferior with the wooden one being the poorest in regard to aspirin stability. The higher the humidity, the larger and quicker the FSA values increased (other than in the glass bottles where little change was found at any of the humidity stations).

Using a Duboscq colorimeter, Tsuzuki and Sawada (47) measured the amount of FSA produced after aspirin had been heated at 110 and 128°. The heated sample (after 5–35 min. at the stated temperature) was dissolved in methanol, the ferric chloride reagent was added, and the solution was compared to standards. The relationship of increased FSA with a corresponding lowering of the melting point of the aspirin was shown.

Pankratz and Bandelin (48) made a systematic and comprehensive study of the optimum conditions for the reaction of ferric iron and salicylic acid and its reproducibility. Maximum absorption of the ferric–salicylate complex in a nearly aqueous medium was at 525 mμ. This complex was very sensitive to pH changes. On studying pH effect at one pH unit increments from 1.0 through 9.0, the maximum color was found between 3.5 and 8.0. Above pH 6.5 the color faded rapidly, so the useful pH range was between pH 4.0 and 6.0. This emphasizes the point that unless the pH of the sample and of the standard series are close, the equivalent

Table IV—Percent Decomposition of Aspirin of Varying Particle Size after 6 Months[a] at 37°

Crystal Size, mesh	Relative Humidity, % 42	59	84
20–50	0.07	0.08	0.16
50–100	0.08	0.09	0.21
100–200	0.08	0.10	0.59

[a] Reprinted, with permission, from R. Yamamoto and T. Takahashi, *Ann. Rep. Shionogi Research Lab.*, 3, 112(1953).

amount of color will not result. One variable, the alcohol concentration, was not controlled. But at the levels used, it apparently did not affect the linearity of the color to concentration. With such a study, one would have expected a comparison of the different ferric salts, particularly those which have been used in the past such as ferric chloride and ferric ammonium sulfate. Instead, this is the first paper in which ferric nitrate was used.

A study involving just pure aspirin, by Yamamoto and Takahashi (49), answered many questions which arise when one seriously wonders under what conditions aspirin is stable or the most stable. The effect of the particle size of the aspirin on its decomposition rate was studied at 37° over a storage period of 6 months at three controlled humidities. The data in Table IV indicate that the finer the crystals and the higher the relative humidity, the more aspirin hydrolyzed. One may also conclude that under 60% relative humidity at 37°, the percent decomposition of aspirin did not change with particle size. With today's use of micronized aspirin, this is a valuable fact.

In studies at 60° for a total of 25 hr. at 30, 60, 80, and 100% humidities, decomposition was linear with time, and the linearity seemed proportional to the vapor pressure because the slope of each line increased with an increase in vapor pressure. Although the FSA content never exceeded 0.04%, this study showed that an increase in humidity did result in an increase in the decomposition rate.

At 90° and at low humidity, the authors found that, after a total of 12 hr., aspirin decomposition was linear with time but had not surpassed 0.1%. At 120° (low humidity), over 15% of the aspirin was decomposed within 5 hr., and the rate of decomposition was no longer linear with time. This study emphasized the fact that temperature increases alone accelerated the decomposition of aspirin.

Grinding of aspirin for 15–120 sec. did not increase appreciably the percent decomposition. On repeated compression (three times) of aspirin, the percent decomposition was found not to have increased appreciably.

In another paper dealing with the stability of aspirin when mixed with other compounds, Yamamoto and Takahashi (50) found that such mixtures should be stored at the lowest practical humidity to prevent excessive decomposition of the aspirin (Table V).

Table V—Stability of Aspirin in Various Powder Mixtures at 37° for 15 Days[a]

Compound Mixed with Aspirin	Ratio of Aspirin to Compound	Percent Loss of Aspirin at 42% Relative Humidity	Percent Loss of Aspirin at 84% Relative Humidity
Antipyrine	10:3	1.2	21.1
Aminopyrine	10:3	8.2	33.2
Hexamine	10:3	56.4	83.4
Ethyl aminobenzoate	10:3	32.6	60.8
Caffeine	10:3	0.01	0.08
Zinc sulfate	10:3	0.02	0.03
Sodium benzoate	10:3	3.2	80.2
Sodium salicylate	100:5	—	26.3
Calcium glycerophosphate	10:3	0.4	4.2
Pheniramine maleate	100:3	—	6.0
Phenindamine tartrate	100:3	—	6.4

[a] Reprinted, with permission, from R. Yamamoto and T. Takahashi *Ann. Rep. Shionogi Research Lab.*, 4, 79(1954).

Table VI—Effect of Ethylenediamine Salts on the Stability of Aspirin[a]

Salt of Ethylenediamine	k_1	Loss of Aspirin, %
Hydrochloride	—	0.6
Maleate	1.0×10^{-2}	3.1
Succinate	6.4×10^{-5}	10.5

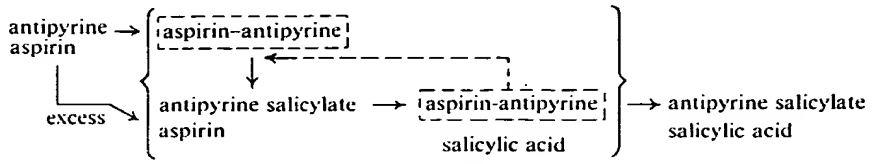
[a] Reprinted, with permission, from R. Yamamoto and T. Takahashi, *Ann. Rep. Shionogi Research Lab.*, 4, 79(1954).

In a comparison of aspirin mixtures with various salts of ethylenediamine, the importance of acid strength on the decomposition of aspirin was shown (Table VI). This study was conducted at 37° and 84% relative humidity for 15 days with a 100:3 ratio of aspirin to ethylenediamine salt.

Further investigation of a 10:1 molar ratio of aspirin–antipyrine mixture stored at 37° and 84% relative humidity for 2 months produced salicylic acid and antipyrine salicylate. The mechanism proposed for this interaction is given in Scheme IV.

Scheme IV was explained in the following manner. First there was an acid–base reaction between aspirin and antipyrine; but because this salt was highly unstable, it decomposed rapidly to antipyrine salicylate. Since aspirin and salicylic acid have approximately the same acidity, the excess aspirin present was capable of reacting with the antipyrine salicylate, releasing salicylic acid and regenerating the unstable aspirin–antipyrine salt which, in turn, started the cycle again.

A similar reaction cycle was proposed for the decomposition of aspirin with pheniramine maleate (amine salt of a weak acid), while no reaction took place with pheniramine hydrochloride (amine salt of a strong acid)



Scheme IV

Table VII—Explanation of Symbols Used in Table VIII

Variable	High Level		Low Level	
	%	Symbol in Table	%	Symbol in Table
Lubricants				
Glycerin monostearate	2	L	0.5	l
Magnesium stearate	2	L	0.5	l
Talc	4	L	1.0	l
Calcium stearate	2	L	0.5	l
Stearic acid	2	L	0.5	l
Mineral oil	4	L	1.0	l
Talc–mineral oil, 1:1	4	L	1.0	l
Pressure	Highest possible	P	Lowest possible	p
Moisture[a]	1.64	M	0.098	m
Aspirin	14-Mesh granules of 10% starch–aspirin granulation	A	40-Mesh crystals	a

[a] Refers to moisture content of the phenacetin–caffeine granulation only.

because aspirin was not capable of displacing the hydrochloric acid.

Without the aid of the computer age, Ribeiro *et al.* (51) undertook a massive, well-executed study of variables in the manufacturing of a stable APC tablet (aspirin, phenacetin, and caffeine). To study the probable causes of the decomposition of aspirin, a factorial experiment was set up testing all combinations of lubricants at two levels, pressure at two levels, moisture at two levels, and two types of aspirin; the entire series was repeated for each of the seven different lubricants (a $2^4 \times 7$ factorial experiment). These variables were selected because they seemed to be the most probable causes of aspirin breakdown in APC tablets. An Association of Official Agriculture Chemists (AOAC) (6th edition) procedure for salicylic acid was modified by using absolute alcohol to extract the salicylic acid from the pulverized tablets and to develop the final iron–salicylate color in a 35% alcohol medium. A spectrophotometer was used to read the resulting color at 537 mμ. Concentrations of salicylic acid were obtained from a standard curve of salicylic acid prepared exactly as the samples. These salicylic acid values were converted to aspirin values and reported in this paper as percent of aspirin content that had decomposed.

Before presenting the data in tabular form, a brief explanation of all the symbols used in the table is essential for proper interpretation of the percent decomposition of aspirin in the tablets after being stored in loosely capped bottles at 45° for 27 days. Tables VII and VIII summarize the results.

Interpretation of these results indicated that more stable combinations were possible if the lubricants used were talc, talc plus mineral oil, mineral oil, or glycerin monostearate rather than stearic acid, magnesium stearate, or calcium stearate. Compression pressure levels showed no effect. Moisture levels were not different enough to be consequential. The crystalline aspirin was superior to the starch–aspirin granulation.

Since difficulties arose in applying the BP colorimetric test for the FSA, Edwards *et al.* (52) initiated an investigation of the kinetics of aspirin hydrolysis and of conditions affecting the formation and stability of the ferric–salicylate complex. Time, as already well known, was an important factor, because aspirin hydrolyzes continually once it is in a given solvent (here alcohol and later water). The need for fast filtration was obvious. Higher temperature expedited the hydrolysis of aspirin, so the lowest practical temperature should be used throughout the test. Constant pH for the test was a

Table VIII—Decomposition of Aspirin after 27 Days at 45° under the Stated Conditions Described in Table VII

Combination	% Aspirin Decomposed[a]						
	Glycerin Monostearate	Magnesium Stearate	Talc	Calcium Stearate	Stearic Acid	Mineral Oil	Talc plus Mineral Oil
MPAL	1.17	8.67	1.10	20.95	8.03	4.11	2.97
MpAL	1.76	8.31	0.97	19.65	8.54	4.59	1.33
MPaL	0.67	7.06	0.55	21.21	0.98	0.99	0.30
Mpal	0.36	2.04	0.00	6.39	0.69	0.39	0.52
MPal	0.33	1.21	0.00	6.95	0.84	0.31	0.22
MpAl	1.63	3.20	2.76	6.20	3.48	4.70	0.87
MpaL	1.05	6.26	0.00	19.54	2.00	0.99	0.75
MPAl	0.99	2.28	2.21	3.84	1.27	2.40	1.16
mPAL	8.56	6.84	1.41	16.04	7.67	1.18	2.66
mpAL	8.59	7.71	2.65	18.35	12.51	2.03	0.58
mPaL	0.96	15.99	0.54	18.54	3.95	0.92	0.87
mpal	0.80	3.39	0.55	3.53	0.44	0.45	0.97
mPal	0.53	3.03	0.48	4.45	1.28	0.90	0.70
mpAl	0.82	2.45	0.49	8.09	2.09	0.68	0.63
mpaL	0.45	15.26	0.73	19.79	4.14	0.93	0.98
mPAl	1.00	3.22	0.73	6.31	3.84	1.06	0.16

[a] The freshly compressed tablets showed negligible decomposed aspirin.

must because the rates of hydrolysis of aspirin vary appreciably with pH. The intensity of the ferric–salicylate complex depends greatly on the pH of the medium. The maximum intensity was obtained between pH 2.5 and 3.5 and was best maintained using an acetic acid–ammonium monochloroacetate buffer. Below or above this pH range the intensity of the color decreased rapidly (intensity at pH 3 was over five times as strong as that at pH 1.5).

As the BP method for FSA is a limit test, a quantitative procedure was developed utilizing the maximum conditions described. If no known interfering materials were present, the aspirin and salicylic acid were dissolved in absolute ethanol diluted with water, all at 25°, and maintained by a thermostated water bath. The time (T_0) was noted when the buffer and ferric ammonium sulfate solution were added and mixed. The volume of the solution was made up to the mark with water, mixed, and kept at 25° for about 10 min. An aliquot was filtered, transferred into an absorption cell, and read at 530 mμ against a reagent blank; the time (T_1) was noted. At least three aliquots were withdrawn at intervals of not less than 10 min. apart. From these values (T_1, T_2, and T_3), a value for the extinction coefficient at zero time (T_0) was obtained by extrapolation. From a standard salicylic acid curve, the amount of salicylic acid at T_0 was thus obtained.

If interfering matter such as phosphate or citrate was present, the aspirin and salicylic acid were extracted from the powdered sample in a separator with benzene. The filtered and pooled benzene was then extracted with small volumes of a solution of the buffer and ferric ammonium sulfate reagent, until an aliquot showed no further coloration in the aqueous extract. The pooled aqueous solution was made to volume with the remaining buffered ferric ammonium sulfate solution and filtered, if necessary, before reading at 530 mμ. The FSA was calculated from a standard salicylic acid curve as before, but without having to calculate a T_0 value; the intact aspirin remains in the benzene layer, so no hydrolysis should be taking place in the aqueous layer.

It is of interest to note that the pH of the aqueous solution (2.95 ± 0.05) read at 530 mμ for both procedures was near the minimum of the hydrolysis rate of aspirin in regard to its pH–rate profile. On comparing the FSA values obtained by using these proposed procedures with those of the BP on several commercial products, the proposed procedure consistently found more FSA, which again raises doubt about the sensitivity of the official BP test for FSA.

Quite independently, Strode *et al.* (53) conducted a systematic study similar to Edwards, only this study involved modifying the USP XV free salicylic test from a limit test to a sensitive, reproducible procedure which was applicable in FSA testing not greater than 0.25%. Hydrolysis curves were constructed from transmittance measurements made at timed intervals on thermostated solutions of aspirin and ferric alum. Under the conditions of the spectrophotometric method, these curves indicated that salicylic acid increased at the rate of 0.0028%/min. at 20°, 0.0036%/min. at 25°, and 0.0054%/min. at 30°. This definitely emphasizes the need for reasonably close temperature control. To obtain reliable readings, 100-mm. cells instead of the usual 10-mm. cells were used. The ferric alum solution in 0.01 *N* hydrochloric acid was kept refrigerated and prepared fresh each week. The calibration standards were prepared so that they would be at the same pH and essentially of the same composition as the sample solutions being measured. This was accomplished by adding an aliquot of freshly prepared aspirin solution in alcohol (SD 30) and water at 25° to individual standard increments of salicylic acid, and noting the time between addition of the ferric alum solution and the reading of final solution (this should be within 5 min.). Through a simplified calculation, a correction for the hydrolysis of the aspirin during this short time interval may be applied. Thus, this is the first time where the salicylic standards contained essentially the same amount of aspirin as the samples being assayed. With colorless aspirin solutions or those from green-tinted formulations, the final iron–salicylate color was read at 515 mμ while the pink-tinted formulations were read at 575 mμ. Appropriate standard curves were run at these given wavelengths. Within the range of concentration measured, the precision and accuracy of this method were within 0.005% salicylic acid at the 95% confidence level.

They also developed a rapid visual method using matched Nessler tubes and a series of salicylic acid standards, which were prepared exactly as the sample in regard to pH, alcohol (SD 30) content, and ferric alum solution. These standard solutions were stable for 2 weeks. The aspirin sample (colorless for this test) was dissolved in alcohol (SD 30), the appropriate aliquot diluted with water cooled to 10°, then treated with the ferric alum solution, and compared within 30 sec. to the standard series of salicylic acid. The salicylic acid content was estimated visually to the nearest 10 mcg. of salicylic acid. By conducting this comparison test so rapidly and at 10°, the hydrolysis error appeared to be within experimental error.

In a thesis and later a publication, Leeson (54) and Leeson and Mattocks (55) made a very thorough study of the decomposition of aspirin in the solid state, utilizing a modification of the AOAC 6th edition procedure which improved the accuracy and sensitivity of the measurements. The aspirin and salicylic acid were dissolved in absolute alcohol. The final color of a given aliquot, which was developed in a 50% alcohol medium, was read in a spectrophotometer at 532 mμ (a slit width of 0.02 mm.) along with a series of salicylic standards treated exactly as the samples.

The step in which the sample or standard salicylic acid aliquot is diluted to exactly 50 ml. with absolute alcohol is an extremely important one. The original procedure consisted of adding the given aliquot to 50 ml. of absolute alcohol, but since the size of the aliquot varied, the concentration of alcohol in the final dilution was not constant. To determine the effect of alcohol concentration on the iron–salicylate color, three different concentrations of salicylic acid were made. They were read over a varying range of alcohol from 5 through 85% alcohol at increasing increments of 10% alcohol. It was readily concluded that the final concentration of alcohol had a significant effect on color intensity. With

Table IX Stability of Various Aspirin–Antacid Mixtures (2:1)

Antacid	Over 1% FSA Stored for Stated Weeks at RT	Over 1% FSA Stored for Stated Weeks at 37.5°	% FSA after 1 Year at RT	% FSA after 1 Year at 37.5°
Dihydroxy aluminum aminoacetate	52+	52+	0.65	0.70
Calcium gluconate	52+	52+	0.80	0.78
Calcium carbonate	28	8	4.4	11.3
Aluminum hydroxide dried gel	12	4	3.9	6.9
Magnesium carbonate	12	2	11.0	42.9
Magnesium oxide	4	2	18.0	24.0
Magnesium hydroxide	2	2	19.5	38.6
Calcium lactate pentahydrate	36	6	71.0	100.0[a]
Magnesium trisilicate	4	2	100.0	100.0[b]
Dibasic sodium phosphate, anhydrous	16	2	100.0[b]	100.0[c]
Sodium bicarbonate	4	2	100.0[b]	100.0[d]

[a] Within 48 weeks. [b] Within 44 weeks. [c] Within 40 weeks. [d] Within 28 weeks.

the lowest salicylic acid concentration, alcohol content of 30 ml. instead of 25 ml. could introduce an error of 6.7% in FSA value. This error increases greatly with higher concentration of alcohol. For this reason, although a worker may select any alcohol volume desired, he must keep it constant throughout the study for both the samples and standards.

Under anhydrous conditions in sealed ampuls, aspirin (100–140-mesh) with and without calcium stearate (a lubricant in aspirin tablets which has been shown to expedite the decomposition of aspirin) were stored at 35, 45, 60, 80, 100, and 110°. Samples were removed at various time intervals over a period of 50 days and assayed for FSA content. Samples of aspirin alone showed little or no decomposition at 80° or below, while those with calcium stearate decomposed within 2 days to the extent of about 1% FSA and then remained near this level during the remainder of the study. At both 100 and 110°, with or without calcium stearate, the aspirin showed about 2% FSA in 5 days and then decreased gradually with time. As these samples both melted and changed color, it was not known whether the formation of a polymolecular salicylide accounted for the decrease in salicylic acid.

From these studies, it was believed that the small amount of decomposition found could have been caused by traces of moisture, which contaminated the dry aspirin during the filling and sealing of the ampuls. The amount of water necessary to account for the decomposition observed was approximately 10^{-5} moles. The conclusion was thus reached that below 80°, the decomposition of aspirin in the absence of moisture was of minor importance.

Consequently, the role of humidity, or more specifically vapor pressure, on the decomposition of aspirin was studied at various temperatures (50, 60, 70, and 80°) with vapor pressures varying from 46 through 232.5 mm. At various time intervals over a period of nearly 1 year, samples were taken from the given humidistats and assayed for FSA content. Decomposition was noted at all stations. The amount depended on the length of time in the given humidistat, temperature, and vapor pressure. The higher the temperature and vapor pressure, the more rapid was the decomposition.

Tablets containing aspirin, starch, and talc (washed and unwashed) were prepared and studied under similar conditions as the aspirin crystals. The effect of washed talc on the stability of the aspirin was not appreciably different from the talc. The complications arose at various humidistats in that the tablets would liquefy, particularly at the 80° stations (but not below 60°), once the salicylic acid content reached a critical level. Once liquefaction occurred, the salicylic acid content decreased sharply and the study with that humidistat was discontinued. Along with previous workers' conclusions, Leeson showed that the compression into tablets did not change the mechanism of decomposition.

The widespread use of aspirin in combination with various antacid compounds as buffering agents led Bandelin and Malesh (56) to study the stability of aspirin with 11 commonly used antacid compounds. Using a modification of the method of Pankratz and Bandelin (48), previously discussed, the FSA content of powder mixtures of two parts aspirin to one part antacid powder, after being stored at room temperature or at 37.5° for periods of time up to 1 year, were assayed at stated time intervals. The antacid powders were used directly from the commercial container so they were not pretreated or dried in any way before using. Table IX summarizes their results.

Both dihydroxy aluminum aminoacetate and calcium gluconate were definitely superior to the other antacids studied in regard to "available" FSA. The word available is used with the FSA reported in that the mixture assayed was extracted directly with acetone. It was not shown, or stated, if aluminum, calcium, or magnesium salicylate was formed during the decomposition of the aspirin, or if the acetone would dissolve these salts. Further, if they did dissolve, would the ferric iron replace the cation in the 50% acetone medium in which the iron–salicylate color was developed?

The unusual was done by Wirth (57) in that he followed the USP XV procedure for FSA *without* any modifications when assaying APC tablets. The FSA values reported were acceptable.

Using the procedure of Ribeiro *et al.* (51), Kral *et al.* (58) studied various mixtures of drugs commonly given with aspirin. Samples of the various mixes were kept at four different stations: room temperature, anhydrous state at room temperature, 97% relative humidity at room temperature, and 37° for periods up to 6 months. The individual mixtures of aspirin with phenacetin, caffeine, phenobarbital, dextrose, sucrose, or lactose were classed as being stable, while those with

Table X—Effect of Amphetamine Salts on the Stability of Aspirin

Amphetamine Salt	pK of Parent Acid	
Picrate	0.38	Increase in accelerating decomposition of aspirin
Acid oxalate	1.19	
Sulfate	1.92[a]	
Acid maleate	2.00	
Acid tartrate	3.02	

[a] Second dissociation constant.

antipyrine, amidopyrine, and quinine hydrochloride decomposed slightly. Mixtures of sodium bicarbonate, hexamethylenetetramine, and caffeine sodium benzoate decomposed rapidly.

In his thesis, Lippmann (59) used the colorimetric procedure developed by Leeson (54), but substituted 95% alcohol for absolute alcohol in his studies of aspirin decomposition with various amphetamine salts. Mixtures of 100 parts of aspirin (50–70-mesh) and 2.935 parts of amphetamine salt as base (60-mesh) were stored in humidity cabinets at 73° and 67% relative humidity. Amphetamine salts used were sulfate, diphenylacetate, *p*-aminobenzoate, 2-naphthoate, phthalate, and picrate. The phthalate salt increased the rate of decomposition of the aspirin the greatest; the order was as follows: phthalate > diphenylacetate = *p*-aminobenzoate = 2-naphthoate > picrate > sulfate.

A relationship between pK of the parent acid in the amphetamine salt and rate of decomposition of aspirin (Table X) correlated well with the acid strength discussed by Yamamoto and Takahashi (50).

Four different tablets of aspirin were manufactured by Nazareth and Huyck (60) to study the effect of calcium salts on the stability of aspirin. The tablets were stored at 9–12, 25–30, and 45° for a period of 8 weeks. Samples were removed weekly and assayed for salicylic acid by essentially the method of Pankratz and Bandelin (48), reading the final color in a 20% alcohol medium. Table XI summarizes their work.

It was noticed that when the percent FSA was over 6, needle-shaped crystals (whiskers) of salicylic acid appeared on the sides and neck of the container. This study showed that aspirin was unstable in the presence of either calcium carbonate or calcium succinate. This was in agreement with Yamamoto and Takahashi (50), who found that the presence of a salt of a weak acid accelerates the decomposition of aspirin.

Continuing the same type of study, Nazareth and Huyck (61) studied the stability of aspirin in four differently manufactured APC tablets. The tablets were stored for 5 weeks and samples removed weekly for the FSA assay.

Only Tablet A in Table XII showed a rapid decomposition of aspirin. It was the only tablet containing magnesium stearate as a lubricant.

As DeMarco and Marcus (62) did not require the sensitiveness described by Leeson (54), they modified the iron reagent to account for larger amounts of salicylic acid and still adhere to Beer's law (Table XIII).

Reagent No. 1 was used by Leeson and was shown to be very sensitive to the alcohol concentration. Reagent No. 4 was recommended, as it was not only insensitive

Table XI—Stability of Various Aspirin Tablets

Tablet	Weeks of Storage before 0.5% FSA Found at— 9–12°	25–30°	45°	% FSA after 8 Weeks at— 9–12°	25–30°	45°
I. Aspirin	8+	8+	7	0.3	0.4	0.5
II. Aspirin + calcium carbonate	7	2	1	0.5	1.2	14.0
III. Aspirin + calcium succinate	5	1	1	0.6	1.0	88.5
IV. Aspirin + calcium carbonate and succinate	4	1	1	0.6	1.2	96.7

to alcohol concentration changes, but the intensity of the iron–salicylate complex was greatly increased. Reagent No. 7 emphasized the need for acid in the color development medium.

Okano *et al.* (63) conducted a factorial experiment ($2^8 \times 4$) like Ribeiro *et al.* (51) and obtained essentially the same conclusions. Of the lubricants, talc, edible oil, and stearic acid were better than magnesium stearate or calcium stearate at 56°. Room relative humidity caused less decomposition than 84% relative humidity. Storage temperature at 45° caused more aspirin decomposition than room temperature (10–20°). Presence of lactose or diphenylpyraline hydrochloride resulted in an increase in aspirin decomposition. Little effect was noticed on the stability of aspirin, with or without starch, whether it was prism or needle form. The number of times the tablets were compressed at different pressures did not affect the aspirin.

In continuing their studies on the stability of aspirin with other drugs or compounds, Patel and Huyck (64) manufactured aspirin tablets with and without aluminum hydroxide dried gel USP. The tablets were stored for nine weeks and samples removed weekly for FSA assay as previously described.

The results in Table XIV are in agreement with the work reported by Bandelin and Malesh (56).

In two papers, Grabowska (65, 66) reported on the stability of aspirin with other drugs as powders or tablets after a year's storage. No decomposition of aspirin was reported when mixed with caffeine, quinine sulfate, codeine phosphate, phenobarbital, phenacetin, carbromal, urea, or *p*-aminobenzoic acid.

Slight decomposition was reported with quinine hydrochloride or sodium benzoate. The presence of sodium phenobarbital, codeine base, or caffeine sodium benzoate greatly accelerated the decomposition of the aspirin. Four stabilizers, magnesium oxide, aluminum hydroxide, calcium carbonate, and calcium gluconate, were mixed individually with aspirin, sodium phenobarbital, and caffeine mix, and aspirin, caffeine, and so-

Table XII—Stability of Various APC Tablets

Tablet	% FSA after 5 Weeks at— 9–12°	25–30°	45°
A	0.6	1.2	16.0
B	0.1	0.2	1.0
C	0.1	0.2	0.6
D	0.1	0.2	1.3

Table XIII—Effect of Iron Reagent and [illegible]ent Alcohol on the Iron–Salicylate Color

Iron Reagent	ml. Iron Reagent Added/100 ml. Solution	% Alcohol in Final Solution	Adherence to Beer's Law at 532 mμ: mcg. Salicylic Acid/ml.
1. 2% Ferric ammonium sulfate in 0.125 *N* hydrochloric acid	2	50	10–20
2. Same as No. 1	5	50	10–60
3. Same as No. 1	5	10	10–80
4. 1% Ferric chloride in 0.1 *N* hydrochloric acid	5	50	10–30
5. Same as No. 4	5	10	10–80
6. 0.5% Ferric chloride in 0.1 *N* hydrochloric acid	2	10	10–50
7. 0.5% Ferric chloride, no acid	2	10	None

dium benzoate mix. Magnesium oxide was best with the former mix, while aluminum hydroxide, calcium carbonate, or calcium gluconate was effective in stabilizing the aspirin in the latter formulation.

Control methods used in the Australian pharmaceutical industry for FSA in aspirin formulations were described by Green (67). The method of Strode *et al.* (53) was used in determining the FSA content in aspirin and aspirin–starch formulations. The results were in line with the BP FSA limits. If the formulation contained magnesium hydroxide, it was necessary to release the salicylic acid from its magnesium salt by first adding an aqueous acid and extracting the salicylic acid immediately with 1:1 pentane–ether solution. After evaporation of the mixed solvent the residue was dissolved in alcohol and assayed.

The effect of selected USP talcs on the stability of aspirin in tablets was reported by Gold and Campbell (68), utilizing a direct benzene extraction of the pulverized tablets. After vigorous shaking and centrifuging until clear, an aliquot was shaken with a ferric ammonium sulfate reagent. The clear aqueous layer, after centrifuging, was read in a colorimeter at 515 mμ. FSA content was calculated from a standard series of salicylic acid treated as the sample. Three series of tablets were prepared. The first used the talc, as is; the second used acid-washed talc. The third series used the best of the four talcs, Talc A, plus known amounts of various impurities (aluminum silicate, red iron oxide, calcium silicate, and calcium carbonate which were mixed individually with the talc to prepare the aspirin tablets).

The data indicated that the four USP talcs were different in regard to FSA formation after being stored at 40° and 90% relative humidity for 12 weeks (FSA varied from 0.8 to 25.8%). The decomposition, however, did not appear to be directly related to the pH of the talc. The acid washing of the talc before use did improve the aspirin stability greatly, particularly with Talc C. Of the added impurities to Talc A, the presence of aluminum silicate or red iron oxide did not significantly affect the stability of the aspirin, while both the calcium salts (carbonate being the worst offender) influenced appreciably the rate of decomposition of the aspirin.

Table XIV—Aspirin Dosage Form Stability

Tablet	% FSA after 9 Weeks at 10°	% FSA after 9 Weeks at RT	% FSA after 9 Weeks at 37.5°
Aspirin	0.13	0.13	0.16
Aspirin and aluminum hydroxide dried gel	1.16	2.04	3.30

Though the main interest of Troup and Mitchner (69) was on degradation of phenylephrine hydrochloride in tablet formulations containing aspirin, they did assay for FSA according to the Gold and Campbell (68) procedure. For the degradation of phenylephrine in aspirin-containing tablets to occur, the breakdown of the aspirin was prerequisite. This was accelerated greatly by the presence of magnesium stearate as a lubricant in the manufacturing of the tablets. For any one formulation held at elevated temperature (usually 70°), the increase in salicylic acid content plotted against the decrease in phenylephrine content gave a linear relationship.

The effect of four granulating solvents in the manufacturing of aspirin tablets was reported by Trose and Danz (70). Granulations prepared with 95 and 70% alcohol or spiritus gelatine did not increase the decomposition of the aspirin, while the use of 5% gelatin mucilage did. The FSA content was found by extracting the pulverized sample with alcohol, filtering, diluting with water, adding ferric chloride reagent, and reading the resulting color at 530 mμ in a colorimeter. If phosphates are present in the formulation, a preliminary extraction and evaporation must be made with 1:1 ether–petroleum ether mixture.

Jaminet and Evrard (71) evaluated the effectiveness of precirol (a glyceryl palmitostearate), a binder lubricant, in the manufacturing of aspirin tablets. After 3 months at 50° and 80% relative humidity, no FSA was reported from the tablets using this substance. The FSA was determined in the pulverized tablets by first extracting with 1:1 ether–petroleum ether solution, filtering, evaporating, dissolving the residue in alcohol, diluting with water, and adding a ferric ammonium sulfate reagent. The solutions were read at 520 mμ.

The effects of humidity on aspirin, on its mixtures and tablets, with five different fillers were reported by Wisniewski and Piasecka (72). The FSA was determined by dissolving the aspirin in alcohol, filtering if

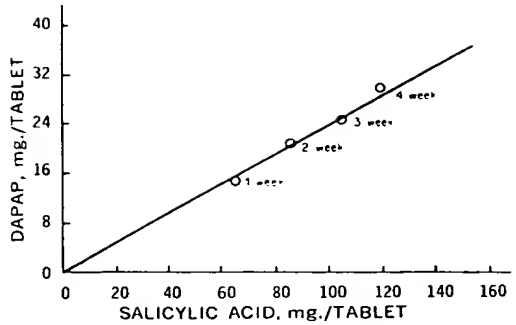


Figure 2—*Relationship between the rate of formation of salicylic acid and of DAPAP.*

required, and adding an aliquot to a Nessler cylinder containing water and the ferric ammonium sulfate reagent. These were compared with the prepared salicylic acid standard solutions. The samples were stored over a period of 4 months at 93, 58, and 20% relative humidity along with changing humidity conditions in the laboratory. The decomposition at 93% relative humidity took place in the following decreasing order of filler used: magnesium oxide > magnesium carbonate > calcium carbonate > magnesium stearate > no filler. This same decomposition order existed at 58 and 20% relative humidity, along with ambient conditions. It is one of the few times that magnesium stearate has not led the list in degree of decomposition of aspirin. During these studies, it was shown that alcohol concentration affected the final color, as did varying amounts of acetic acid. None of the fillers used altered the final color.

In a paper similar to that of degradation of phenylephrine (69), Koshy *et al.* (73) reported on the acetylation of acetaminophen (APAP) in tablet formulations containing aspirin. With tablets stored at 50° for a period of 6 weeks, the formation of diacetyl-*p*-aminophenol (DAPAP) indicated a linear relationship between the rate of formation of salicylic acid through a 4-week period (Fig. 2). The same trend existed in various samples stored at ambient conditions, although at lower salicylic acid and DAPAP levels. Commercial tablets of unknown age, purchased from retail outlets and containing both APAP and aspirin, showed this same relationship of aspirin decomposition and formation of DAPAP. As one of these lots showed high DAPAP, a study was conducted at 50° for 4 weeks on the effect of stearic acid in one and magnesium stearate in the other. The magnesium stearate mix produced nearly 1000 times as much DAPAP as the stearic acid or regular mix.

Continuing their study on the stability of aspirin, Jaminet and Louis (74) made tablets with various lubricants. On assaying these tablets after being stored 6 months at 50° and 80% relative humidity, the lubricants may be listed in order of increased amounts of salicylic acid being found. Magnesium stearate was the poorest lubricant in that nearly 90% of the aspirin was decomposed, the next being polyethylene glycol 6000 (about 15% being decomposed) > glycerol monostearate II > stearic acid > stearyl alcohol > glycerol monostearate I > precirol = geleol. The last two lubricants were the best in protecting the aspirin from decomposing under these conditions.

Table XV—Effect of Common Active and Inactive Aspirin Product Components on Color Development[a]

Component	Component–Salicylic Acid Ratio	Interference
Lactose	40:1	Nil
Meprobamate	100:1	Nil
Methylcellulose	40:1	Nil
Polyvinylpyrrolidone	20:1	Nil
Phenacetin	60:1	Nil
Calcium stearate	10:1	+20%
Magnesium stearate	10:1	+20%
Stearic acid	10:1	+30%
Alginic acid	10:1	Nil
Ion-exchange resin[b]	10:1	Nil
Caffeine	40:1	Nil
Ethoheptazine citrate	20:1	Nil
	100:1	−15%
Citric acid	15:1	−20%
Dihydrocodeine	20:1	Nil
Codeine phosphate	20:1	Nil
Phenergan HCl	3:1	+100%
Hydrogenated vegetable oil[c]	10:1	Nil
Talc	20:1	Nil
Meperidine HCl	40:1	Nil
Starch	70:1	Nil

[a] Reprinted, with permission, from L. F. Cullen *et al.*, *Ann. N.Y. Acad. Sci.*, 153, Art. 2, Table 1(1968). [b] Amberlite, Rohm & Haas Co. [c] Sterotex.

It is befitting that the last paper using an iron reagent would be titled "An Automated Colorimetric Method for Determination of Free Salicylic Acid in Aspirin-Containing Products," by Cullen *et al.* (75). The number of salicylic acid determinations required in the development of a stable aspirin product, or in production quality control, can be overburdening. The need to automate the salicylic acid analysis was, and is, great, as the saving in analytical time, effort, manpower, and expense can be tremendous.

The method selected for automation was that of Pankratz and Bandelin (48), as they had reported on the optimum conditions needed in order to obtain the greatest accuracy and reproducibility in the quantitative determination of salicylic acid in pharmaceutical preparations. A flow diagram (Fig. 3) is presented at this time only to show the equipment arrangement utilizing a standard Technicon automated system which was programed at 15 samples/hr. Specificity studies are summarized in Table XV.

Turbidity accounted for the high values given by the presence of calcium and magnesium stearate and stearic acid. The quenching effect (negative interference) was brought about by citric acid which forms a nonionized salt with iron. Phenothiazine derivatives react directly with the ferric iron and so must be removed if present in the aspirin formulation. Linearity of the salicylic acid in the microaperture flow-through cells was excellent. The precision of standard salicylic acid showed a repeatability deviation of 1.5%, while replicate samples of a commercial tablet were within 2%. Good accuracy was demonstrated by recovery of known amounts of standard salicylic acid added to powdered commercial tablets and by comparing this to the manual procedure.

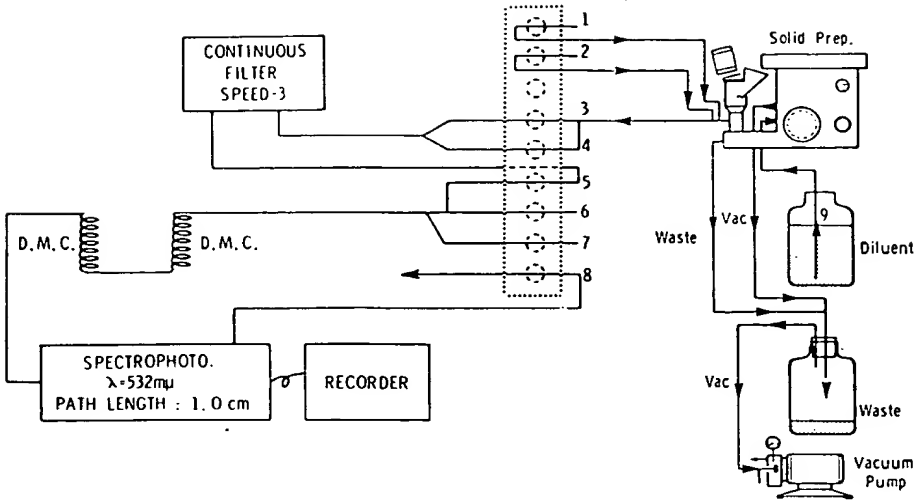


Figure 3—*Flow diagram for the determination of salicylic acid in aspirin-containing products. Key: 1, 0.8 ml./min. air; 2, 4.06 ml./min. SDA No. 30 alcohol; 3, 2.76 ml./min. sample; 4, 2.03 ml./min. sample; 5, 2.76 ml./min.; 6, 2.76 ml./min. $Fe(NO_3)_3$ reagent; 7, 2.00 ml./min. air; 8, 2.50-ml./min. flowcell; and 9, reservoir-SDA No. 30 alcohol.* [*Reprinted, with permission, from L. F. Cullen* et al., Ann. N. Y. Acad. Sci., **153**, *Art. 2, Fig. 1 (1968).*]

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS BY UV SPECTROPHOTOMETRY

Until Tinker and McBay (76) introduced their simple and rapid spectrophotometric method of analysis for aspirin and salicylic acid, two separate determinations had to be made for the intact aspirin and hydrolyzed aspirin. In selecting the solvent for the UV studies, they found that in using chloroform A.R. grade, both aspirin and salicylic acid had higher absorbances and stabilities than in either aqueous or alcohol solutions. Also, the greatest difference between maximum and minimum absorbance values for any given concentration was observed in chloroform. The maximum absorbance for aspirin and salicylic acid was found to be 278 and 308 mμ, respectively, as shown in Fig. 4. Beer's law was conformed to in the concentration ranges used. Equations were developed for this two-component mixture, and the application to aspirin, aspirin tablets, or capsules was valid and had an error of less than 0.2%. Samples of aspirin were dissolved in chloroform (filtered if necessary) and read at 308 mμ for salicylic acid content. A portion of the bulk solution was diluted 100 times and then read at 278 mμ for aspirin content. Both readings were against a chloroform blank.

Ebert (77) found that the existing methods for determining amphetamine sulfate, phenacetin, and aspirin in the corresponding tablets involved many laborious time-consuming extractions. After the separations were completed, lengthy titrations or a gravimetric procedure were required. With all this work, very little, if any, reliable information was available in regard to subtle changes such as the presence of salicylic acid which could be formed from the decomposition of the aspirin. Ebert, with meticulous care, designed a three-component spectrophotometric method for the simultaneous determination of aspirin, phenacetin, and salicylic acid. It was not only a more rapid method of analysis, but was found to give increased accuracy and precision. Alcohol was selected as the solvent of choice. In this medium, aspirin was found to have an absorption maximum at 226 mμ, salicylic acid at 235 mμ, and phenacetin at 250 mμ. As there was a great deal of overlapping of the three absorption curves, it was important to prove that the absorbance of this mixture, particularly where the aspirin showed 0, 50, and 100% hydrolysis, represented the sum of the absorbances of the individual compounds comprising the mixture at that wavelength. The validity of this was well proven by Ebert's work. From all these basic data, the appropriate validated equations were derived. The equations were then tried on tablets which had been extracted with ether. The three compounds in alcohol were then determined spectrophotometrically. The estimate of the accuracy and precision compared favorably with those methods used in the past. Though the major emphasis of this work was on the stability of sympathomimetic amine salts when combined with aspirin and phenacetin, interesting facts evolved about the stability of aspirin in these combinations. It might be added that in all the stability studies conducted here, phenacetin did not show any appreciable change.

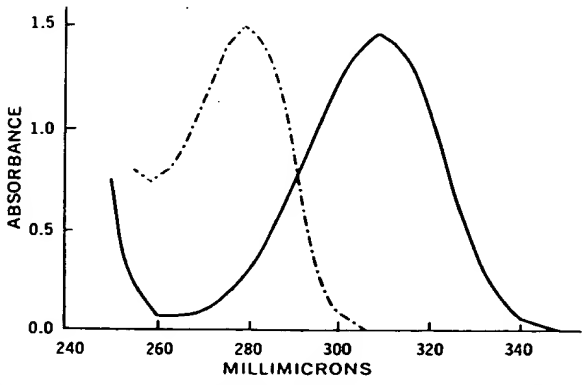


Figure 4—*UV spectrocurves of aspirin (- - -) and of salicylic acid (——) in chloroform.*

Stability studies on tablets containing amphetamine sulfate, aspirin, and phenacetin were conducted in regard to the effects of temperature, moisture, and lubricants on these compounds. All the tablets were packaged in loosely capped amber bottles. These bottles were stored under the following conditions: room temperature, 0% relative humidity; room temperature, 95% relative humidity; 43°, 0% relative humidity, and 43°, 95% relative humidity. After a year's storage and many assays, the important finding was that aspirin decom-

position appeared to be associated with the disappearance of the amphetamine sulfate. The aspirin decomposition appeared to be dependent upon the lubricant present in the following decreasing order: magnesium stearate > talc and magnesium stearate > stearic acid > talc. The increase of temperature accelerated the decomposition of aspirin, but the increase of humidity at the same temperature had a much greater effect. Methamphetamine hydrochloride was substituted for the amphetamine sulfate in a similar stability study, and the conclusions were the same as mentioned for amphetamine sulfate. It is of interest to note that no aspirin decomposition occurred in any of the formulas stored at room temperature, 0% relative humidity, regardless of the ingredients present. In another study, salicylic acid replaced aspirin in the tablet with the result that it had little or no effect on the amphetamine sulfate.

Ebert checked on the possibility that aspirin might be capable of acetylating amphetamine. Working with the pure compounds, he isolated acetylamphetamine which proved this belief. The addition of an equivalent amount of salicylic acid and acetic acid in place of the aspirin in that study did not result in the isolation of any acetylamphetamine. Isolation of any acetylamphetamine from the tablets studied was not successful. Today, with the aid of TLC and GLC, such a decomposition product could readily be detected quantitatively.

Though Leeson's work with aspirin (54) involved chiefly a colorimetric procedure, he used the UV procedure developed by Ebert. This was done to check that no loss of salicylic acid (through volatilization) from the vials on stability had occurred. As the tablets used here did not contain amphetamine sulfate or phenacetin, the interpretation required was simpler. Absolute alcohol was used in place of the 95% alcohol. This would assure better stability of the aspirin during the assay. Readings were taken at 226 mμ (slit width of 0.8 mm.) and at 235 mμ (slit width at 0.6 mm.) against absolute alcohol. On assaying many tablets randomly, the summation (in moles) of the aspirin and salicylic acid found accounted for all the aspirin originally employed. In short, no salicylic acid was being lost through volatilization under the storage conditions.

In the first of a series of many interesting and valuable publications dealing with the determination of the stability of aspirin in many products, Levine (78) introduced a rapid chromatographic assay for APC tablets which included the determination of salicylic acid. Previous papers using this column technique did not discuss the determination of salicylic acid.

The pulverized APC sample was extracted with chloroform containing a small amount of acetic acid (to convert any aspirin salt to the free acid and so be extracted by the chloroform). Without filtering off the insoluble excipient material, an aliquot was diluted with freshly water-washed ether and passed directly to the single duplex column which had just been washed using water-washed ether. The upper segment of the column contained 1 *N* sodium bicarbonate on diatomaceous earth[4] which acted as the supporting phase. The sodium bicarbonate trapped both the aspirin and salicylic acid. The lower segment of the column contained 4 *N* sulfuric acid on diatomaceous earth. The sulfuric acid retained the caffeine while the phenacetin passed through into an evaporating dish. Water-washed ether was passed through the column in small portions to elute quantitatively all the phenacetin. A volumetric flask was placed under the column and sufficient water-washed chloroform was passed through the column to elute quantitatively the caffeine. Immediately, the column was eluted with acetic acid in chloroform (previously water-washed before adding the acetic acid) into another volumetric flask. This last eluate was read immediately in a suitable spectrophotometer at 280 mμ for aspirin and 310 mμ for salicylic acid, as aspirin was not stable in this medium. The caffeine was read at 276 mμ while the phenacetin was evaporating. The residue in the evaporating dish was dissolved in a little chloroform and then diluted with isooctane in a volumetric flask and read at 285 mμ.

This entire procedure, from grinding of the tablets to the final UV readings, took less than 1 hr. Though it was not emphasized, the aspirin should not be left on the column any longer than necessary, as it has been found to hydrolyze readily. From experience it has been found that the phenacetin may be dissolved and diluted with chloroform; thus another solvent (isooctane) is unnecessary.

In calculating, standards of the three drugs and salicylic acid are read in the UV at the stated wavelength in the same medium as the sample. For practical purposes, it was assumed that aspirin's absorbance at 310 mμ was negligible (100 mcg. aspirin/ml. read 0.010). Thus, any reading at 310 mμ was calculated as salicylic acid. From this absorbance value at 310 mμ, the absorbance due to the salicylic acid at 280 mμ can be calculated from the values of the standard salicylic acid read at these two wavelengths and deducted from the total absorbance at 280 mμ; the remainder can be considered to be the absorbance of the intact aspirin.

This partition chromatographic procedure compared excellently with the old and very laborious NF X procedure. The fact of the matter is that Levine's work was so convincing (and deservedly so) that in the next revision of the NF (NF XI) his procedure replaced the old official method except for the FSA limit test. There were also other firsts in this paper; Levine broke tradition with conventional partition chromatography techniques by altering the nature of the immobile phase (neutralizing the sodium bicarbonate phase of the column with acetic acid) as a step in the process. In so doing, the versatility of partition chromatography was thus broadened to permit separations not previously possible. In earlier techniques, the constitution of the immobile phase remains unchanged.

In 1959 and 1960, Smith (79, 80) reported on an AOAC collaborative study of the Levine method for APC. As a result of these studies, the Levine method became official in the Tenth Edition of the Methods of Analysis of the AOAC. These collaborative tests did show a weakness in the procedure, in that the values reported for the salicylic acid content varied appreciably (from 0.15 to 3.17% as hydrolyzed aspirin). As stated before, with care the hydrolysis of the aspirin on the

[4] Celite 545, Johns-Manville Corp., New York, N. Y.

sodium bicarbonate column can be avoided, chiefly by eluting the column as quickly as possible. Smith suggested that the aspirin and salicylic acid content could be calculated by simultaneous equations rather than make the assumptions already discussed in the Levine paper.

Heuermann and Levine (81) and later Heuermann (82) expanded the usefulness of Levine's original work to the analysis of combinations of aspirin, phenacetin, and caffeine with other drugs. The other drugs were pyrilamine maleate, chlorprophenpyridamine maleate, phenindamine tartrate, methapyrilene hydrochloride, doxylamine succinate, thonzylamine hydrochloride, codeine sulfate or phosphate, phenobarbital, or cyclopentylallybarbituric acid. In neither paper were there any salicylic acid values reported, although discussion was made of its determination. An absorbance figure greater than 0.020 at 310 mμ indicated that partial hydrolysis of the aspirin had taken place and must be accounted for in the calculations of the aspirin content of the sample. Simultaneous equations were used in determining the intact aspirin and the FSA content of the sample.

In the application of his partition chromatographic procedure to just aspirin and aspirin tablets, Levine (83) required only the sodium bicarbonate segment of the column. With this paper, he clarified the salicylic acid status in that if 5% or more FSA was found, the aspirin and salicylic acid contents of the given sample were found simultaneously by the described UV procedure. If the FSA was under 5%, another technique was used. (This technique will be described later in this Review Article.)

With the UV procedure, it was noted earlier that aspirin was not stable in chloroform so a little acetic acid was added to acidify the medium. However, in the case of buffered tablets, a stronger acid was needed, not only to stabilize the aspirin but to protect it from the hydrolytic effect of the buffering agents present. This was accomplished by preparing a 0.24 *N* hydrochloric acid solution in methanol and adding a small amount of this solution to the original extraction medium of chloroform. The amount of acid present did not affect the efficiency of the chromatographic column, even if the tablets being assayed were not buffered. An aliquot of the aspirin solution was passed through the column with the aid of more chloroform (no ether was required as with APC tablets). The aspirin was eluted with acetic acid in chloroform and determined at 280 mμ, while the reading at 310 mμ showed the salicylic acid content. If the absorbance at 310 mμ was 0.075 or higher, it represented a concentration of 5% or more FSA. The aspirin reading at 280 mμ was corrected for this FSA absorbance so that the intact aspirin could be reported. If the FSA was below 5%, another method was used in reporting FSA since this spectrophotometric procedure resulted in too low an absorbance reading to be accurate. This procedure was successfully applied to regular aspirin tablets (white), pink aspirin tablets, orange-colored and flavored children's aspirin tablets, enteric-coated tablets, buffered aspirin tablets, and aluminum aspirin tablets.

The stability of aspirin compounded with 10 different kinds of antacids was reported by Kubo *et al.* (84). The samples were stored for a total of 90 days at: 5°, 52% relative humidity; 20°, 75% relative humidity; and 30°, 92% relative humidity. Samples were removed intermittently for assay by a UV spectrophotometric assay. The FSA content was calculated directly from the value at 308 mμ, while the intact aspirin value at 275 mμ had to be corrected for the salicylic acid absorbance value. The antacids studied were aluminum silicate, magnesium carbonate, magnesium trisilicate, calcium gluconate, calcium lactate, sodium phosphate, dried aluminum hydroxide gel, calcium carbonate, magnesium oxide, and sodium bicarbonate. Only the 5° station showed good stability with these antacids. At the 30° station, the last four mentioned antacids were completely incompatible with aspirin, as shown by the high FSA values.

Even though pyrilamine was known to interfere with the salicylic acid reading at 308 mμ, Siegel *et al.* (85) used the Tinker and McBay (76) UV procedure to expedite the analysis of tablets of pyrilamine resin adsorbate with aspirin and ascorbic acid. Tablets were stored at 60° for 1 week and for a total of 12 weeks at 45°. Samples removed at various time intervals showed an unexpected trend because the type of container closure and degree of fill were of prime importance for evaluating these products by accelerated temperature studies. Tablets stored in open bottles, or those with polyethylene snap caps, had greater stability than those with Bakelite screw caps. Filled containers appeared to have greater instability than partially filled ones; bottles that had been opened frequently, compared to those opened only once, appeared more stable. The following explanation was offered by the authors: the passage of air would remove moisture as well as gaseous acidic degradation products which promote instability in these tablets; thus, in tightly closed and well-filled bottles, this would not take place to as large a degree.

Chapman and Harrison (86) determined FSA in soluble aspirin tablets by dissolving the aspirin in glacial acetic acid, filtering, and reading the absorbance at 320 mμ against glacial acetic acid. The salicylic acid content was found from a standard calibration curve of salicylic acid run exactly as the sample. This solvent was selected because aspirin was not stable in chloroform for UV studies, and the results obtained were more reproducible than the BP procedure.

A more thorough study of the Tinker and McBay (76) procedure was reported by Ladomery (87). Chloroform was replaced with absolute spectral alcohol as the solvent for aspirin and salicylic acid. At the wavelength of maximum absorbance for salicylic acid, 300 mμ, Beer's law held through 80 mcg./ml. of absolute spectral alcohol. Similar equations were calculated, only using the absorption data acquired from the alcohol medium. Application of this method was acceptable and accurate.

For APC preparations which contain both barbituric acid derivatives and certain organic bases, it was the objective of Turi (88) to develop a single method rather than using two procedures as described by Heuermann and Levine (81). He was successful with capsules

or tablets containing aspirin, phenacetin, caffeine, itobarbital, and one of four phenothiazine derivatives (chlorpromazine hydrochloride, promethazine hydrochloride, thiethylperazine dimaleate, or thioridazine hydrochloride). No mention, however, was made of determining salicylic acid by this column procedure other than that a prolonged stay (on Column III) would result in a partial *in situ* degradation of the aspirin.

For the determination of aspirin, caffeine, and acetaminophen (APAP), Koshy (89) found that the reversal of the column arrangement described by Heuermann and Levine (81) was all that was required. The three active ingredients could be assayed beside the potential decomposition products, salicylic acid and *p*-aminophenol. After the two columns were prepared in the usual manner and placed in tandem, such that the top column contained the sulfuric acid as the immobile phase and the bottom column contained the sodium bicarbonate as the immobile phase, they were both washed with ether. The powdered sample was dissolved in ethyl acetate, an aliquot of which was then passed through the two columns and collected in a volumetric flask. The columns were further eluted with ether. This fraction contained intact APAP. The columns were then eluted with chloroform. This fraction contained the caffeine. The two columns were then separated, and the bottom column (sodium bicarbonate phase) eluted immediately with acetic acid in chloroform. This fraction contained the aspirin and salicylic acid and was assayed in the usual manner. The top column (sulfuric acid phase), containing *p*-aminophenol, was washed with ether to remove the chloroform. The diatomaceous earth support was then extruded from the column with air under pressure and collected in a beaker. The ether was evaporated from this material, and 0.1 *N* hydrochloric acid was added to dissolve the *p*-aminophenol. This extract was filtered and an aliquot was assayed colorimetrically using 1-naphthol as described by Greenberg and Lester (90).

The effect of water vapor pressure on moisture sorption and the stability of aspirin and ascorbic acid in tablet matrixes reported by Lee *et al.* (91) utilized the Tinker and McBay (76) procedure in evaluating the stability of the aspirin in these studies. Conclusions reached from these studies showed that the moisture adsorptive capacity of each compressed tablet formulation affected the stability of the two drugs to a great extent and were directly related to the moisture sorption and tablet hardness. (The harder the tablet, the less moisture it sorbed and the more stable the drug.) Of the six diluent systems studied, calcium sulfate and cellulose produced the most stable tablets of aspirin and ascorbic acid, while amylose produced the least stable. Under stress-storage conditions, screw-cap glass bottles proved to be a better moistureproof container than snap-top plastic vials. Cellophane and aluminum foil strip packaging materials were about equally effective. Both were more effective than the glass or plastic containers.

Reed and David (92) described a simple direct spectrophotometric determination of salicylic acid in either one complete capsule or one intact tablet of an aspirin containing medicinal, provided no interference by the other components with the salicylic acid absorption at 300 mμ was encountered. The entire dose unit is shaken with alcohol for 1 hr. along with a similar freshly prepared unit dose as a "blank" (as this was generally not available, an equivalent amount of fresh aspirin was weighed and used as the blank). This exposure to an alcoholic medium for 1 hr. could lead to generated hydrolysis in the case of aspirin tablets containing buffers as there is no mention of pH control here or in the aqueous dilution being read at 300 mμ. The sample was read against the blank so that the effect of the intact aspirin would be cancelled out, provided the difference between the two concentrations was not great.

Application of the Tinker and McBay (76) procedure was applied by Day *et al.* (93) in following the stability of two mixtures official in the BPC. They reported the accuracy of the method to be ±2.5%.

In a general paper on the use of UV for analysis of drugs in pharmaceuticals, Sattler (94) applied the method described by Ladomery (87) which was a modification of the Tinker and McBay procedure.

A direct spectrophotometric determination of five compounds, aspirin, salicylamide, caffeine, phenacetin, and salicylic acid, in tablets or powders without any preliminary separation was reported by Clayton and Thiers (95). The powdered sample was extracted with chloroform. From this extract, three aliquots (same volume size) were added to separate volumetric flasks, one to be an acidic medium, another to be a basic medium, and the third a hydrolyzed medium. A mixed solvent consisting of isopropanol, water, and a small amount of hydrochloric acid was added to each flask followed by a solution of 50% sodium hydroxide to the basic and hydrolyzed labeled flasks. After hydrolysis of the aspirin was complete at room temperature (about 15 min.), concentrated hydrochloric acid was added to the hydrolyzed labeled flask, rendering it acid again. On diluting all flasks to volume with the mixed solvent, the solutions were then read at the following wavelengths along with the appropriate reference blank solution. The acidic labeled solution was read at 250, 273, and 310 mμ, the basic labeled solution at 333 mμ, and the hydrolyzed labeled solution at 301 mμ. Using a variety of equations, the content of the individual components was calculated. The comment was made that if salicylic acid were absent, it was possible to omit one step, but the omission is not recommended, since this step provides a measure of any hydrolysis of aspirin which might have occurred during storage or manufacture of the product analyzed.

Use of the isosbestic point as a base line in differential spectrophotometry was applied to aspirin and salicylic acid by Shane and Routh (96). When a series of concentrations of salicylic acid were used to prepare differential absorption spectra, monosodium salicylate (at pH 9) in the reference cell *versus* disodium salicylate (at pH 13.5) in the sample cell, two maxima at 246 and 319 mμ, two minima at 233 and 203 mμ, and two isosbestic points at 268 and 300 mμ were observed. If differential absorption spectra of aspirin solutions were prepared by the same procedure (monosodium acetylsalicylate in the reference cell *versus* disodium salicylate equivalent to the monosodium acetylsalicylate in the sample cell), a

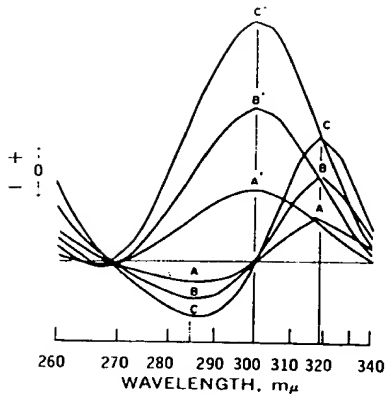


Figure 5—*Differential spectra of salicylic acid (A, B, C) and aspirin (A′, B′, C′). [Reprinted, with permission, from N. A. Shane and J. I. Routh,* Anal. Chem., *39, 414, (1967).]*

maximum at 300 mμ, a minimum at 268 mμ, and an isosbestic point at 272 mμ were observed. Figure 5 illustrates the differential absorption spectra of three concentrations of salicylic acid (A, B, and C) compared to the spectra of three concentrations of aspirin (A′, B′, and C′) in the region from 260 to 340 mμ. Each curve is constructed from the two spectra of the same concentration of either salicylic acid or aspirin obtained at pH 9 and 13.5 by subtracting one spectrum from the other spectrum, resulting in the differential absorption spectrum of the given compound. The isosbestic point in differential spectrophotometry would be the wavelength at which each difference curve crosses the zero line. The correlation of the isosbestic point at 300 mμ for salicylic acid, and the maximum at the same wavelength for aspirin, permits the use of the isosbestic point as the zero or base line for the quantitative determination of intact aspirin in the presence of salicylic acid. Thus, one would not need to do a FSA determination as the value reported for aspirin is for the intact aspirin.

A very much needed publication on the significance of salicylic acid sublimation in stability testing of aspirin-containing solids was presented by Gore *et al.* (29). A more than negligible loss of salicylic acid formed from the decomposition of aspirin would preclude the common practice of analytically determining changes in salicylic acid content in solid dosage forms of aspirin as a measure of degrading aspirin. The salicylic acid method could obviously underestimate the extent of decomposition of aspirin and, therefore, provide false confidence in the stability of the tested products. It thus became necessary to develop a method of gauging aspirin stability in solids which would be unaffected by any loss of salicylic acid.

A simultaneous spectrophotometric assay, based chiefly on the work of Edwards (8), was developed for aspirin and salicylic acid in a Clark and Lubs buffer, pH 7.4. It was found that this aqueous medium resulted in improved precision over the chloroform medium used by earlier workers. This slightly alkaline medium afforded a relatively rapid solution of solid aspirin and salicylic acid, and a medium in which slight variation in the pH would not introduce an error into the determination as a consequence of the differential absorption of ionized and unionized aspirin or salicylic acid. The rate of hydrolysis of aspirin has been reported (8) to be independent of pH in the range of 4 through 8. Hydrolysis rate constants were determined at pH 7.4 and 25.5°, and showed a delay of 13 min. between the sample preparation and reading on the spectrophotometer would cause an error of approximately 1%. The actual error was reduced to below 0.1% by maintaining the solutions below 15°, usually at 0°, and reading them within 5–10 min. of their preparation.

Table XVI presents the pertinent data required in the construction of the calibration curves used here.

Before applying this UV procedure, several sublimation studies were conducted in which the need for such an assay was demonstrated quite convincingly. Using an electrobalance, the weights of salicylic acid were monitored continually at the stated temperatures for 12 hr. From this study the following rates of sublimation were calculated (Table XVII).

An Arrhenius-type plot of the apparent zero-order sublimation rates was shown. The slope of the curve was predominately determined by the enthalpy of sublimation of salicylic acid. The observed rates of sublimation may be expected to depend directly upon the area through which the mass transfer occurred. These results, therefore, were not intended to be quantitatively indicative of sublimation loss of salicylic acid during the stability testing, but merely substantiate that such a loss can occur even at moderately elevated temperatures.

A similar sublimation study of purified aspirin revealed no significant loss of weight up to 70°. It was concluded that aspirin does not appreciably sublime under the conditions of the experiment.

With a 9:1 mixture of aspirin–salicylic acid, the results from 12 hr. at 70° indicated that only salicylic acid was lost. This was verified by using the UV procedure discussed.

Aspirin tablets were stored at 50° and 81.2% relative humidity for a period of 98 days. At various time intervals tablets were removed and assayed by first grinding to a fine powder and dissolving in pH 7.4 buffer maintained at 0°. After filtration, an aliquot of the filtrate was further diluted with the cold pH 7.4 buffer and the absorbance measured at 262 and 296.5 mμ. In this application, an error of 0.003 absorbance unit at 262 mμ could contribute an error of approximately 1% in the

Table XVI—Constants for Aspirin and Salicylic Acid UV Assay

Compound	Concentration Range, mcg./ml. of pH 7.4 Buffer	Absorptivity Value at 262 mμ	Absorptivity Value at 296.5 mμ
Aspirin	0–160	3.2	0
Salicylic acid	0–10	3.3	—
Salicylic acid	0–30	—	26.0

Table XVII—Sublimation Rates of Salicylic Acid at Various Temperatures

Temperature	Rate of Sublimation, mg./hr.
40 ± 0.05°	0.026
50 ± 0.20°	0.062
70 ± 0.20°	0.372

Table XVIII—Comparison of Aspirin Tablet Stability Testing Results at 50° and 81.2% Relative Humidity, Based on Determination of Aspirin and Salicylic Acid Content of the Tablets[a]

Time, Days	Aspirin Content Based on Analysis of Aspirin, %	Aspirin Content Based on Analysis of Salicylic Acid, %	Error Due to Sublimation of Salicylic Acid, %
0	100	100	0
15	99.1 ± 0.038	99.1 ± 0.028	0
30	98.4 ± 0.042	98.8 ± 0.215	0.4
45	97.2 ± 0.123	98.7 ± 0.075	1.5
60	97.1 ± 0.178	98.6 ± 0.023	1.5
98	95.0 ± 0.288	97.9 ± 0.311	2.9

[a] Each value is the average of four determinations recorded with ±1 standard deviation.

determination of the aspirin content of a solid consisting of 80% aspirin using a 50-mg. aliquot for analysis. The results of this study are presented in Table XVIII.

The last column does show that salicylic acid is lost from these tablets by sublimation under the stated conditions of the experiment.

By employing the UV procedure presented in this publication, the determination of the residual aspirin, rather than the apparent salicylic acid, in a solid can be used as a valid means of gauging the stability of the formulation. This method of analysis showed an accuracy within at least 1.5%.

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS WITH THE AID OF A FERRIC-ION CHROMATOGRAPHIC COLUMN

Analytically, the need has always been great to separate and isolate the salicylic acid from aspirin as well as other components in the pharmaceutical preparation. The potential susceptibility of aspirin to hydrolysis is constantly prevalent, so the more rapidly the intact aspirin is removed from the medium, once a given procedure is begun, the truer the reported FSA values will be.

Again Levine (83) appeared to be first in breaking away from the traditional methods of determining salicylic acid in aspirin formulations. On using the chromatographic columns he had introduced in 1957 with a diatomaceous earth–2% ferric chloride mix, the passage of a chloroform solution of aspirin and salicylic acid resulted in the trapping of the salicylic acid (shown by a purple zone on the column). The aspirin passed through, and by using several washes with chloroform, the aspirin was completely removed from the column. The bound salicylic acid was then eluted quantitatively with chloroform containing acetic acid. This eluate was read at 310 mμ for FSA. For the procedure to be valid, the purple zone should not reach the bottom of the tube during the washing out of the aspirin. If it does, the procedure must be repeated with another prepared column. As the original procedure was written, this was a major downfall in using this technique, because too many times the purple zone moved partially off the column during the aspirin removal step.

This procedure was applied to pink aspirin tablets, children's flavored and colored aspirin tablets, buffered aspirin tablets, and aluminum aspirin tablets. With the colored tablets, the dyes remained at the top of the column throughout the entire assay. With the buffered tablets, the chloroform-insoluble salts of aspirin or salicylic acid must be transformed to soluble acids in order that the FSA could be eluted in the proper fraction. Acetic acid could not be used since it would dissociate the ferric ion–salicylic acid complex on the column. Boric acid, however, was used for this transformation without affecting the column performance. A solution of boric acid in methanol was added to the sample, followed by chloroform to dissolve the freed aspirin and salicylic acid.

For enteric-coated tablets it was found best to mount in tandem a column containing just diatomaceous earth above the regular ferric ion–diatomaceous earth column. The plain diatomaceous earth column removed the surface-active agents present in these tablets, so the aqueous phase would not be stripped off the regular ferric ion–diatomaceous earth column during both the prewashing step and elution of the salicylic acid. This double column setup could be used also where dyes are present in the original chloroform extract, as well as for large amounts of excipient material in the sample being added to the column.

As will be seen with later papers dealing with this novel approach of Levine's, a delicate balance was being maintained with the ferric chloride content on the diatomaceous earth. A sufficiently low concentration of ferric chloride must be maintained to trap the salicylic acid, but at the same time a sufficiently large quantity of ferric chloride must be present to provide an excess over the amount removed during the washing step. It is thus recommended that the modifications of this procedure, to be discussed in this review, be used rather than the method described in this publication. Just the introduction of this approach to the literature served a very worthwhile purpose as the official compendia now use a procedure based on this original study.

Green (67) applied this described procedure by Levine and as he did not present any comments in his paper, it could be assumed that he did not experience any major difficulty with the columns.

In 1961 Weber and Levine (97) made note that "several investigators have encountered difficulty with the published method" (83). During the elution of the aspirin, the salicylic acid migrated slowly down the column (as evidenced by the position of the purple complex) and spread out into a diffuse band, which sometimes becomes difficult to discern.

In this publication, this was rectified by modifying the ferric chloride reagent. This radical improve-

ment in the chromatographic separation was achieved by having a high concentration of urea in the ferric chloride solution. The resultant effects were tremendous: the band of the ferric-salicylate complex obtained with this new reagent was more deeply colored than that obtained with just the simple ferric chloride reagent. The dense, sharply delineated band migrated only slightly during the elution of the aspirin.

It, therefore, became feasible to use a shorter column which did not require extraordinary care in packing. This column even accommodated larger samples of aspirin.

Optimum conditions were obtained with an immobile phase containing 5% ferric chloride solution which was 10 *M* with respect to urea. The pH must be maintained between 3.1 and 3.3. At lower pH levels the salicylic acid band became diffuse and more loosely retained, while at higher pH levels, recovery of salicylic acid from the column may be incomplete using the specified volume of eluant.

For aspirin or aspirin tablets, the pulverized sample was dissolved with chloroform and passed through the column with the aid of several more portions of chloroform to wash the intact aspirin through the column. A volumetric flask containing some hydrochloric acid in methanol was placed under the column, and the column first eluted with acetic acid in ether followed by chloroform. Concomitantly the absorbance of this solution and of the standard salicylic acid in the same medium was determined at 306 mμ.

For APC tablets and flavored tablets, a column containing a small pad of cotton was placed in tandem over the regular ferric chloride–urea column. The sample in the chloroform was passed first through the cotton column to remove the dyes and any insoluble excipients. Another portion of chloroform was passed through the two columns, and then the top column was discarded. The regular column was then washed with chloroform and the salicylic acid eluted.

As moderate amount of urea was eluted together with the salicylic acid from the column, the hydrochloric acid in the receiving flask was added to maintain acidity of the eluate. The methanol was present to achieve miscibility of the acid with the eluting solvent.

With buffered aspirin tablets, the boric acid was replaced with oxalic acid in methanol, as the oxalic acid was more effective in releasing the bound salicylic acid from the antacid. The oxalic acid solution recovered quantitatively salicylic acid from its calcium salt. However, neither this nor any other reagent thus far tested quantitatively released salicylic acid from aluminum hydroxide gel without causing extensive hydrolysis of aspirin.

Excellent reproducibility on replicate analyses of a wide variety of commercial samples was shown.

Though Weber's publication (98) on the analysis of salicylic acid and benzoic acid does not deal with aspirin, *per se*, it had a valuable innovation regarding the ferric chloride–urea–diatomaceous earth column. When eluting salicylic acid of larger quantities than usual, the occasional leakage of ferric chloride was noted which invalidated the assay. This was seen visually by the presence of a yellow color rather than the usual colorless clear eluate. If any yellow is present in the solution read, the reading at 306 mμ would have to be voided. Weber found that a small layer of diatomaceous earth with 30% phosphoric acid as the immobile phase placed at the bottom of the column on top of the glass wool support, and then followed by the regular ferric chloride–urea–diatomaceous earth mix, resulted in no ferric chloride leakage. The phosphoric acid retained any ferric chloride which might be removed during the elution step of the salicylic acid.

In their study on the formation of acetylcodeine from aspirin and codeine, Jacobs *et al.* (99) used the original Levine procedure (83) in isolating the salicylic acid which resulted from the previously mentioned reaction.

On discussing pharmaceutical heterogeneous systems, Zoglio *et al.* (28) have presented four papers regarding the hydrolysis of aspirin. The salicylic acid resulting from the degradation of aspirin in the various formulations was determined by a modification of Levine's iron–diatomaceous earth procedure (83). The first paper [Kornblum and Zoglio (28)] is discussed in the *Hydrolysis Studies* portion of this review. They demonstrated that calcium or magnesium stearate accelerated the production of salicylic acid from aspirin through the solubilization of aspirin as a calcium or magnesium salt. More aspirin would thus be in solution which in turn would hydrolyze in the existing pH which was conducive to hydrolysis. This effect was not as pronounced when aluminum stearate was used as a lubricant, as the aluminum salt of aspirin has a low solubility in water.

On pursuing this alkali stearate effect, Zoglio *et al.* (28) prepared capsules containing aspirin (20 parts), magnesium stearate (1 part), and 0, 1, 2, 5, 10, and 20 parts of hexamic, maleic, malic, or tartaric acids, or maleic anhydride. These capsules were stored at 22, 40, and 50° for 30 days. A minimum of 20% by weight of hexamic, maleic, or malic acid was required to retard the hydrolysis of aspirin in these capsules. Tartaric acid or maleic anhydride was not effective at the higher temperatures. Besides the lower desired pH contributed by the added acid, the mechanism of inhibiting the degradation of aspirin involved the additive acid and aspirin competing for the magnesium ion.

Maulding *et al.* (28) showed that stearic acid USP, which is nearly a 1:1 mixture of stearic and palmitic acids, promoted the decomposition of aspirin more than either reagent grade stearic or palmitic acid. On preparing various synthetic mixtures of reagent stearic and palmitic acids, the one simulating the amounts of the two acids in stearic acid USP behaved similarly as the stearic acid USP in accelerating the decomposition of aspirin. This ratio of stearic and palmitic acids was also the melting point minimum of the various mixes of these two acids. The possibility exists, therefore, of a liquid or semiliquid being present in formulations containing stearic acid USP which might serve as the medium for aspirin hydrolysis.

The fourth paper dealt with the acceleration of aspirin hydrolysis by various common additives (hexamic acid, aluminum hydroxide calcium stearate, magnesium stearate, or magnesium trisilicate), at a 5 or 10% level of the powder mix or tablet. The formulations con-

taining magnesium trisilicate resulted in the largest amount of FSA (about 13%) after 45 days at 40°. Hexamic acid, under these storage conditions, retarded aspirin hydrolysis. The salicylic acid values resulting from these various formulations were in good agreement with values obtained from extrapolating apparent zero-order rates of aspirin suspensions of the same powder mix as used in the tablet. These studies show that stability prediction for solid dosage forms from apparent zero-order rates of suspensions is feasible and informative.

The determination of the FSA in buffered aspirin tablets by Levine and Weber (100) explored further the usefulness of their ferric chloride–urea–diatomaceous earth column. It was essential that the entire amount of aspirin and salicylic acid be dissolved in the mobile phase before the chromatographic treatment, and that the aspirin was not hydrolyzed during the preparation of that solution. These two requirements are not readily achieved in the case of buffered aspirin tablets. Chloroform solutions of aspirin, in the presence of basic materials such as those which comprise the buffering components of the tablets, undergo hydrolysis together with aspirin anhydride formation. Boric acid stabilizes the solution, at least with respect to the hydrolysis of aspirin, but does not achieve the necessary release of the aspirin and salicylic acid from the buffer components to permit their complete solution in chloroform.

In designing a valid assay procedure, the acid which is used must: (*a*) produce only minimal hydrolysis of aspirin under the conditions of the assay; (*b*) rapidly and completely release aspirin and salicylic acid from the buffer components of the tablet; (*e*) be soluble in chloroform; and (*d*) be readily removed from the chloroform, so that the ferric–salicylic acid complex will not be dissociated during the following step of the analysis.

These requirements were fulfilled by 98–100% formic acid. The distribution of formic acid between chloroform and inorganic acids was greatly in favor of the aqueous phase; therefore, the formic acid was removed from the chloroform solution by passage over dilute hydrochloric acid.

In the analysis of buffered aspirin tablets, two columns are prepared and placed in tandem. The top column consists of diatomaceous earth with 0.05 *N* hydrochloric acid as the immobile phase. It is here that the formic acid in the chloroform is removed. The packing of this column should be such that a flow rate of chloroform of 12 ml./min. is obtained.

The bottom column consists of a layer of diatomaceous earth with 30% phosphoric acid as the immobile phase. The phosphoric acid retains any ferric iron which may be eluted during the procedure. The upper stage of this column consists of the 5% ferric chloride and 10 *M* urea as the immobile phase on diatomaceous earth.

In the procedure, water-saturated solvents were used throughout. To a ground sample of tablets in a volumetric flask, the 98% formic acid was added with swirling to wet the sample completely (not more than 30–45 sec.) followed by chloroform. This mixture was shaken for 10 min. The extent of hydrolysis of aspirin during the period of contact of the sample with the formic acid before dilution lies in the range of 0.01–0.02%/min., so here the extent of hydrolysis will be in the order of 0.01%. After dilution with the chloroform, the hydrolysis of aspirin sharply decreased to an average of only 0.03%/hr. Thus a negligible amount of hydrolysis occurs during the 10-min. shaking period for dissolving the aspirin and salicylic acid. On diluting to volume with chloroform and mixing, the solution was filtered through a loose plug of glass wool. An aliquot of this filtrate was then passed through the double columns which were washed with more chloroform to remove the aspirin. The eluate and the top column were discarded. A receiver containing hydrochloric acid in methanol was placed under the remaining (bottom) column, and ether containing acetic acid was passed through the column followed by chloroform containing acetic acid. This eluate was read at 306 mμ along with a salicylic acid standard in the same medium.

By this method, great strides have been made in regard to freeing salicylic acid from various antacids. It was shown that the formic acid treatment readily recovers salicylic acid from its calcium or magnesium salts but that aluminum salicylate was quite refractory to this treatment. As dried aluminum hydroxide gel is present in several of the commercial buffered aspirin tablets, the need is still acute for a method which will recover salicylic acid from its aluminum salt.

On studying the USP XVII limit test for salicylic acid in aspirin tablets containing buffers, Guttman (101) obtained spurious and nonreproducible results. Low recoveries were explained by an adsorption phenomenon. Significant adsorption of salicylic acid occurred when solutions of chloroform were in contact with chloroform-insoluble agents which are commonly employed as buffers in aspirin tablets. The affinity of salicylic acid for magnesium carbonate and aluminum glycinate was high, but the capacity of these solids for the acid was rather low. These two compounds held tenaciously to the salicylic acid, as it was impossible to elute completely adsorbed material by repeated contacts with fresh solvent.

High recoveries of salicylic acid resulted from a surprisingly rapid transformation of aspirin to a product having the chromatographic characteristics of salicylic acid. This was observed only when solid basic material (here magnesium carbonate) was suspended in the chloroform solution of aspirin. This transformation of aspirin was thus surface catalyzed.

On repeating this experiment with different commercially available buffered aspirin products, the same phenomenon occurred except with one—and it contained citric acid monohydrate besides two antacid compounds. With this sample, the salicylic acid content did not increase appreciably with time of contact with the solid suspension. With all these suspension studies, it was of interest to note that, upon filtration, immediate cessation of salicylic acid production resulted. This was shown by the readings in the UV at 278 and 306 mμ on the chloroform filtrate or when the ferric chloride–urea–diatomaceous earth column procedure (USP XVII) was used.

Table XIX—Results Obtained when Various Methods Were Employed to Estimate Salicylic Acid (SA) Contents of a Number of Commercial Buffered Aspirin Products

Product	Buffers Present	% SA Found Using: USP Procedure	Citric Acid Procedure for FSA	Weber and Levine (97) Procedure	Citric Acid Procedure for Total Nonaspirin Salicylates
1	Aluminum hydroxide Magnesium hydroxide	2.19 2.80 3.03	4.02 4.02 3.89	6.59 6.53	6.64 6.58 6.62
2	Aluminum glycinate Magnesium carbonate	Not detectable	0.211 0.236 0.219	0.64 0.69 0.62 0.67	0.728 0.718 0.739
3	Aluminum hydroxide Glycine Magnesium carbonate	0.455	1.01	3.79 3.91 3.52	3.78 3.58 3.58
4	Calcium phosphate Sodium bicarbonate Citric acid	—	5.22	—	5.92
5	Aluminum hydroxide Glycine Magnesium carbonate	—	0.682	—	2.81
6	Aluminum hydroxide Magnesium hydroxide	—	1.82	—	3.39
7	Calcium carbonate Magnesium carbonate	—	1.96	—	3.37

On further studies with aspirin in chloroform and magnesium carbonate in suspension with and without an equal weight of citric acid monohydrate, it was found that no salicylic acid was produced in 200 min. at 25° with citric acid present. After 60 min. of magnesium carbonate being in contact with the aspirin solution, the addition of citric acid monohydrate resulted in no further salicylic acid production. It was visually observed that the addition of citric acid had a pronounced effect on the nature of the suspension. Marked flocculation of particles was apparent immediately after the addition of citric acid monohydrate. Citric acid which had been previously oven-dried showed a much less marked effect in inhibiting salicylic acid production. This showed that presence of water from the hydrate was essential in this reaction with suspended material. Applying this citric acid monohydrate to the previously studied commercial buffered tablets on an equal weight basis showed that in all systems studied here, the citric acid was effective in markedly reducing the catalytic ability of suspended solids in the production of salicylic acid from the aspirin in chloroform.

Repeating the magnesium carbonate–aspirin adsorption experiment discussed earlier, only this time with and without an equal amount of citric acid monohydrate, it was found that in the presence of citric acid there was no appreciable adsorption of the aspirin from the chloroform. It is also of interest that treatment of antacid compounds in suspension in chloroform with citric acid monohydrate significantly reduced their capacities for adsorbing salicylic acid.

Guttman's study demonstrated very convincingly that finely divided solids of antacids do catalyze a conversion of aspirin to a product which, by the analytical method employed, was determined as salicylic acid. The reaction occurred under essentially anhydrous conditions, and adsorption of the aspirin was apparently a prerequisite for the transformation. The presence of citric acid monohydrate was shown to be effective in inhibiting the formation and adsorption of salicylic acid in the systems reported here. A feasible explanation was given: citric acid monohydrate, which is essentially chloroform insoluble, inhibits the reaction by releasing water of hydration to the surface of the adsorbent. A neutralization reaction takes place in the hydrate layer which modifies the surface characteristics of the adsorbent so as to destroy the adsorption sites. Thus, the catalytic production of salicylic acid from aspirin is never initiated.

Results from studies presented in this paper showed that addition of citric acid monohydrate to the official chromatographic procedure in the original extraction of the sample resulted in almost quantitative recovery of salicylic acid. It was also noted that this citric acid treatment was not effective in displacing salicylic acid from metallic salts which are known to form in buffered aspirin products.

In applying the procedure suggested in the preceding paper, Guttman and Salomon (102) compared its usefulness (and superiority) over the existing USP XVII FSA test for buffered tablets of aspirin. The method consisted of treating, by trituration, a powdered sample with an equal weight of citric acid monohydrate, and dissolving the aspirin and the FSA from the powder mass with chloroform. The remaining residue was treated with an aqueous solution of a strong acid (hydrochloric acid), and this solution was extracted with chloroform. The two chloroform extracts (one containing the FSA and the other containing the nonaspirin salicylates) were combined, and the salicylic acid content determined by the chromatographic method of Weber and Levine (97). With the assumption that the citric acid treatment results only in desorption of salicylic acid and aspirin and does not cause conversion of salicylate salts to free acid, one would have a method available for the estimation of the FSA content

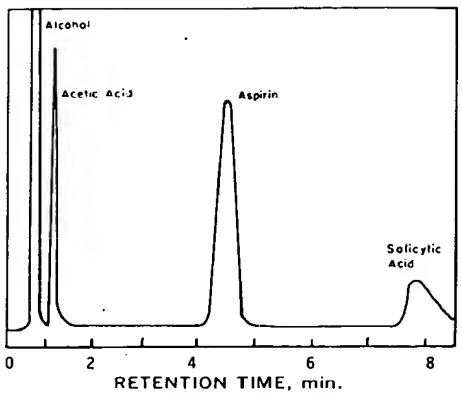


Figure 6—*Gas chromatogram of aspirin in alcohol. [Reprinted, with permission, from J. G. Nikelly,* Anal. Chem., **36**, *2248 (1964).]*

as well as total nonaspirin salicylate contents of buffered tablets of aspirin. Table XIX summarizes the results on applying four different procedures to commercially available buffered aspirin tablets: USP XVII (using boric acid in the initial extraction), Guttman's citric acid procedure, Guttman and Salomon's citric acid procedure for total nonaspirin salicylates, and the Levine and Weber procedure (100) which used formic acid in the initial extraction medium.

Inspection of this table makes obvious that the FSA values, as determined by the USP procedure, were consistently and significantly lower than those determined by the other methods. It is logical to assume that these low values resulted from adsorption of significant amounts of salicylic acid during sample extraction, and to the insensitivity of the procedure to salicylic acid which is present in the sample in the form of salts. The latter two methods appear to be equally precise. It is interesting to note the difference between the citric acid procedure for FSA and the citric acid procedure for total nonaspirin salicylates. The differences reflect the fact that significant amounts of salicylic acid can be present in buffered tablets as chloroform-insoluble salts. It is also apparent that no relationship exists between the FSA content and total nonaspirin salicylate content of these tablets.

DETERMINATION OF SALICYLIC ACID IN ASPIRIN AND ASPIRIN PRODUCTS BY MISCELLANEOUS METHODS

Schulek and Burger (103) stated that on using BrCl as a brominating agent, salicylic acid could be brominated quantitatively in 2 min., even in the presence of aspirin. With the usual amounts of FSA present in aspirin products, it is debatable whether such a titration would be sensitive enough to be of value.

The application of differential thermal analysis (DTA) and thermogravimetric analysis (TGA) to aspirin was published by Wendlandt and Hoiberg (104). No mention was made of the effects of the presence of salicylic acid, but application of this approach would definitely be of interest in evaluating the purity of aspirin.

Gas chromatography has a great potential in the analysis of aspirin products, but only two papers have mentioned the possibilities. Crippen and Freimuth (105) stated that their proposed procedure would differentiate aspirin from salicylic acid only if the temperature of 100–125° and a 1.23–1.83-m. (4–6-ft.) column were used. Nikelly (106) showed (Fig. 6) that under the stated conditions used in the determination of aspirin, the amount of acetic and salicylic acids could be estimated.

In applying an IR spectrophotometric assay of aspirin anhydride, Garrett and Johnson (107) concluded that the insignificance of salicylic acid in these lots of varied purity showed that little definition was lost on ignoring it. Though samples of aspirin were not used here, the usefulness of IR studies on the purity of aspirin in regard to salicylic acid would be of value.

The need for actual physical separation of salicylic acid from aspirin is great. The work by Vietti-Michelina (108) and Wagner (109) on paper chromatographic or paper electrophoretic separation of aspirin and salicylic acid might be applied successfully by using the latest techniques available in this field.

A too often neglected technique in drug studies in the United States is the application of polarography. In 1949 Korshunov *et al.* (110) reported on the reduction of weak acids at a dropping-mercury cathode. The half-wave potentials were given as 1.52 to 1.65 (for 1–7 mmoles/l.) for aspirin and 1.66 to 1.83 (for 1–9 mmoles/l.) for salicylic acid in 0.05 *N* tetramethylammonium iodide. With the more sensitive polarographs of today, such an approach in the analysis of aspirin products could prove to be useful.

Quantitative separation of aspirin and salicylic acid by sephadex G gel filtration was reported by Lee *et al.* (111). No detectable (fluorometrically) hydrolysis of aspirin on the column was noted.

TLC should be an invaluable tool in aspirin stability studies. By using a mixture of hexane, glacial acetic acid, and chloroform (20:5:5), Reimers (112) separated aspirin and salicylic acid. After resolving the mixtures of aspirin–salicylic acid on thin-layer plates [using ascending technique and a hexane, glacial acetic acid, and chloroform mixture (85:15:10), R_f values of 0.2 and 0.35 were obtained for aspirin and salicylic acid, respectively], the spots were removed from the dried plates, packed in an appropriate cell, and determined by UV reflectance spectroscopy. A linear relationship at 302 mμ between absorbance and the square root of the concentration was observed by Frodyma *et al.* (113) with spots containing up to 3.0 μmoles of either compound.

An exhaustive paper on differentiating nonaqueous titration of salicylic acid and aspirin by Lin (114) showed that such a procedure was feasible. Dimethylformamide was the solvent of choice along with the titrant of 0.1 *N* tetrabutylammonium hydroxide in benzene–methanol (10:1) using either a glass–calomel or platinum–calomel electrode pair.

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RESEARCH ARTICLES

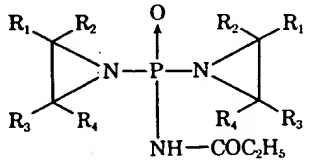
Mechanisms of Reactions of Ring-Substituted Bis(1-aziridinyl)phosphinyl Urethan Antineoplastic Agents

C. K. NAVADA, Z. F. CHMIELEWICZ, and T. J. BARDOS*

Abstract □ Bis(*trans*-2,3-dimethylaziridinyl)phosphinyl urethan (IV) was synthesized and compared with the corresponding *cis*-2,3-dimethyl derivative (III). The comparative alkylating activities and rates of hydrolysis of these two stereoisomeric aziridine derivatives, III and IV, were determined and compared with corresponding data for the monomethyl derivative (V) and two other clinically tested members of this series of antineoplastic agents (dual antagonists), AB-100 (I) and AB-132 (II). The structures of the final hydrolysis products of III, IV, and V were determined and confirmed by direct synthesis. The results indicate that the mechanisms of hydrolysis of III, IV, and V (as that of the unsubstituted aziridine derivative, AB-100) are essentially S_N2, in contrast to the much faster hydrolysis of the 2,2-dimethylaziridine analog, AB-132, which involves a carbonium-ion mechanism. These studies give further support to the hypothesis that the unique pharmacologic properties of AB-132, as compared to other members of this series, may be related to the unique chemical properties of the 2,2-dimethylaziridine moieties.

Keyphrases □ Antineoplastic agents—reaction mechanisms □ Bis(*trans*-2,3-dimethylaziridinyl)phosphinyl urethan—synthesis □ Alkylating activity—*cis*-, *trans*-2,3-dimethylaziridine analogs □ Hydrolysis mechanism—ring-substituted aziridine derivatives □ IR spectrophotometry—identity □ NMR spectroscopy—identity

The synthesis of a series of bis(1-aziridinyl)phosphinyl carbamates, termed "dual antagonists" (I and its analogs containing different carbamate moieties) (1, 2), and their antineoplastic activities in experimental animals (3, 4) and in man (5–9) were previously reported. In an effort to decrease the hematologic toxicity due to the "alkylating" aziridine groups, derivatives were synthesized in which the C-atoms of the aziridine rings were substituted with methyl or ethyl groups (10). One member of this new series, ethyl bis(2,2-dimethyl-1-aziridinyl)phosphinyl carbamate (AB-132, II), has been studied to a considerable extent experimentally (11) as well as clinically (12–17). Its interesting pharmacologic properties [*e.g.*, cholinesterase inhibition (18–20)] and its radiation potentiating effect (21–23) suggested that this compound may act by a different mechanism than the C-unsubstituted aziridine derivatives (24). This conclusion was supported by chemical studies of its hydrolytic and alkylation reactions (11, 25), which indicated that the unique properties of II may be related to the ability of the 2,2-dimethylaziridine group to participate in S_N1 reactions with its substituted carbon (by forming a tertiary carbonium ion) and, alternatively,



I. $R_1 = R_2 = R_3 = R_4 = H$ (AB-100)
II. $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$ (AB-132)
III. $R_1 = R_3 = CH_3$; $R_2 = R_4 = H$ (*cis*) (AB-144)
IV. $R_1 = R_4 = CH_3$; $R_2 = R_3 = H$ (*trans*) (AB-145)
V. $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$ (AB-143)

Exhibit H

Effect of Silica Gel on Stability and Biological Availability of Ascorbic Acid

E. De RITTER, L. MAGID, M. OSADCA, and S. H. [illegible]

Abstract ☐ The alleged interaction of silica gel and ascorbic acid has been investigated in model experiments and in practical tablet trials, using wet granulation procedures. In simple mixtures stored for 3 weeks at 45° in closed tubes, losses of ascorbic acid increase progressively with increasing moisture content, whether or not silica gel is present, although losses are higher in the presence of silica gel. At an equivalent percentage of water in such mixtures, the amount of silica gel or the prior adsorption of 1½ times its weight of vitamin E on the silica gel, did not influence the loss of ascorbic acid. The data show that silica gel binds a certain fraction of the water present and that the loss of ascorbic acid is directly proportional to the amount of unbound water in the system. Sodium ascorbate is more sensitive than ascorbic acid to aerobic oxidation in the presence of moisture. Other commonly used tablet excipients, as well as silica gel, enhance losses of ascorbate. However, proper technology applied to wet granulation procedures yields excellent recoveries and stability of ascorbic acid or sodium ascorbate in dried granulations and in finished multivitamin tablets. The human bioassay technique, in which extra urinary excretion of ascorbic acid after tablet dosage is compared to that after dosage of ascorbic acid in water, has been used to demonstrate the full physiological availability of ascorbic acid in the presence of silica gel. Storage of such tablets for 3 months at 45° did not alter the complete bioavailability of the ascorbic acid.

Keyphrases ☐ Ascorbic acid—stability, biological availability ☐ Stability, ascorbic acid—humidity, excipient effects ☐ Silica gel effect—ascorbic acid stability, bioavailability ☐ Moisture concentration—ascorbic acid stability ☐ Biological availability, ascorbic acid—silica gel effect

Diffuse reflectance spectroscopy has been used by Lach and Bornstein (1–3) to study interactions of a number of drugs with various adjuvants after treatment of the mixtures by equilibration in aqueous or nonaqueous media, by compression, and by exposure to controlled humidity conditions. Such an interaction of ascorbic acid and silica gel has been claimed by Lach (4). Since silica gel is a useful adsorbent for converting liquid vitamins such as vitamin E and panthenol into free-flowing, dry powders, it became important to evaluate this alleged interaction of silica gel with ascorbic acid. This has been done in model experiments with simple mixtures and under practical conditions of formulating multivitamin dosage forms. In addition, physiological availability tests in humans have been utilized to check for possible influence of silica gel contained in multivitamin tablets on the biochemical behavior of ascorbic acid.

EXPERIMENTAL

Model Experiments—(*a*) *Effect of Graded Moisture Levels*—Experimental mixtures of ascorbic acid with silica gel[1] and with 60% adsorbate of *d,l*-α-tocopheryl acetate on silica gel were prepared both at normal use ratios and at an eightfold higher than normal adsorbent/vitamin ratio. Ascorbic acid alone and the various mixtures were adjusted with distilled water to graded moisture levels up to 40% and stored in closed tubes for 21 days at 45°. The percentage of water added was based in each case on the total weight of the tube contents, except for the vitamin E adsorbate mixtures where the weight of the oil phase was not included. The compositions of the mixtures before addition of water are shown in the legend of Fig. 1. Ascorbic acid was determined after storage by titration with about 0.1 *N* standard iodine solution and starch indicator.

(*b*) *Rate of Loss of Ascorbic Acid and Sodium Ascorbate at 45° with 11.6% Water*—The stress conditions used in *Experiment* (*a*), namely 3 weeks at 45° at high moisture levels, are obviously much more strenuous than those normally encountered in pharmaceutical manufacturing operations, such as wet granulation procedures, in which drying is completed in a much shorter period. It was of interest, therefore, to check the rate of decomposition of ascorbic acid in similar mixtures stored for 1, 2, and 3 days at 45° at one of the lower levels of moisture, namely 11.6%.

Ascorbic acid and the ascorbic acid plus silica gel mixture used in this test, when shaken with water at a concentration of about 20 mg. of vitamin C per ml., yielded a pH of 2.3 and 2.4, respectively. Sodium ascorbate with and without silica gel, at similar dilutions in water, gave a pH of 6.7 with silica gel and 7.2 without. The same stability tests were set up with sodium ascorbate with and without silica gel at 11.6% water.

(*c*) *Effect of Other Tablet Excipients*—The relative effect of other excipients commonly used in tablets has been compared to that of silica gel in a test similar to that in (*b*). Three hundred milligrams of sodium ascorbate were mixed with 80 mg. of the particular excipient and water added to give 11.6% by weight. The mixtures were stored in closed tubes for 3 days at 45° and ascorbate determined by iodine titration.

Granulation and Tablet Trials—Multivitamin mixtures containing ascorbic acid or sodium ascorbate and silica gel adsorbates of vitamin E were made by wet granulation procedures with and without iron. To minimize exposure to moisture stress, the granulations were milled through a No. 6, round-hole screen to the minimum practical particle size and dried in layers of 1.27 cm. (0.5 in.) or less with rapidly moving, 45° air. Vitamin C recoveries were determined for the granulations and finished tablets made from these granulations, using iodometric titrations. Stability of vitamin C was determined similarly after accelerated and room temperature storage.

Availability Studies in Men—It has been pointed out (1–3) that drug-adjuvant interactions possibly may result in significant altera-

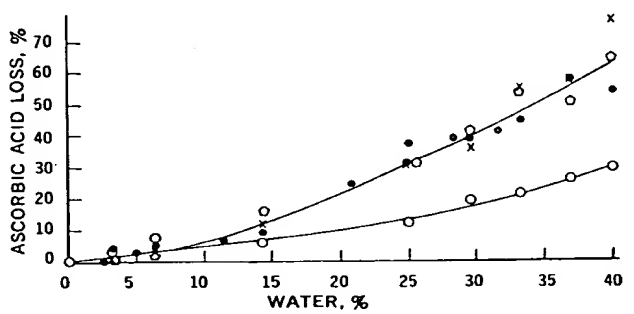


Figure 1—*Effect of silica gel on stability of ascorbic acid at graded percent moisture levels; mixtures stored in closed tubes for 3 weeks at 45°. Key:* ○, *300 mg. ascorbic acid alone;* ●, *300 mg. ascorbic acid + 80 mg. silica gel;* ⌓, *300 mg. ascorbic acid + vitamin E adsorbate (80 mg. silica gel + 120 mg. d,l-α-tocopheryl acetate);* ×, *300 mg. ascorbic acid + 640 mg. silica gel.*

[1] Syloid 244, W. R. Grace & Co., Davison Chemical Div., Baltimore, Md.

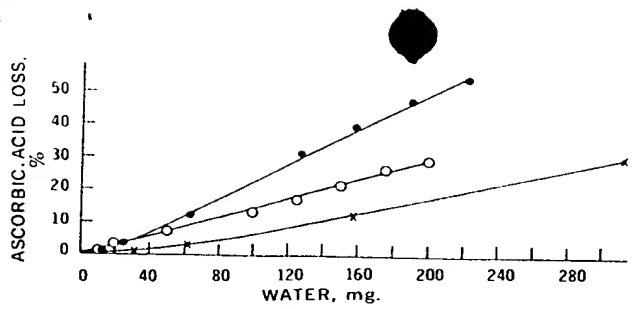


Figure 2—*Effect of silica gel on stability of ascorbic acid with graded weights of water added; mixtures stored in closed tubes for 3 weeks at 45°. Key: ○, 300 mg. ascorbic acid alone; ●, 300 mg. ascorbic acid + 80 mg. silica gel; ×, 300 mg. ascorbic acid + 640 mg. silica gel.*

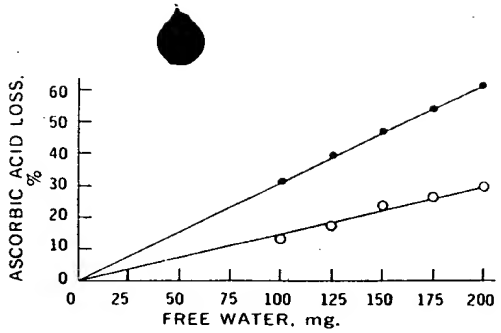


Figure 3—*Effect of free water level on stability of ascorbic acid in mixtures with or without silica gel; storage in closed tubes for 3 weeks at 45°. Key: ○, 300 mg. ascorbic acid alone; ●, 300 mg. ascorbic acid + 80 or 640 mg. silica gel.*

tions of the biochemical behavior of a medicament. Although such effects are more likely to occur at low drug to adjuvant ratios, the possible existence of the excipient as a chemisorbed layer on the drug has been mentioned as a factor that might cause similar effects at high drug to adjuvant ratios (2). To determine whether silica gel in a tablet would influence the physiological availability of ascorbic acid, the human bioassay technique described by Melnick *et al.* (9) was applied to several tablet formulations. In this test, comparison is made between the extra urinary excretion of the vitamin following dosage with the test sample and that following administration of the vitamin in pure form.

Five male subjects were saturated with ascorbic acid by daily dosing with 500 mg. for 3 weeks. Dosing with test samples and standard was not initiated until a stable plateau had been obtained for the 24-hr. urinary excretions following a 500-mg. dose, given after 2 days without dosing. Two different tablet formulations as listed in Table VI were tested, both containing vitamin E at a level of 30 mg. per tablet in the form of silica gel adsorbate. The standard dose of pure ascorbic acid taken in water was 450 mg. For the initial test, the dose taken was six tablets (453 and 466 mg. ascorbic acid for the Lot Nos. 73-69/1 and 73-69/3, respectively). For the aged sample, seven tablets (430 mg. ascorbic acid) were given.

One test was performed each week with basal urine collected on the day prior to taking each test dose. After dosage with ascorbic acid alone or in tablets, respectively, urine was collected for the periods 0–6 hr. and 6–24 hr., except for Dose 2 of ascorbic acid where only total 24-hr. collections were made. Basal urines were collected in each case according to the same schedule. Ascorbic acid in urine was determined by the dichlorophenol–indophenol–xylene extraction method, as previously described (10). In each case the extra excretion due to dose was calculated by subtracting the corresponding basal excretion value from the value after dose.

RESULTS AND DISCUSSION

Model Experiments—*Effect of Graded Moisture Levels*—The results of storage tests on the ascorbic acid plus silica gel mixtures at graded moisture levels for 3 weeks at 45° are shown in Fig. 1. Ascorbic acid alone shows progressively increasing storage losses with increasing moisture content. At low moisture levels (below 6%), no significant difference could be determined between samples with or without silica gel. At higher moisture levels, the losses increase with increasing moisture content in mixtures containing silica gel and are higher than those found with ascorbic acid alone. It is noteworthy, however, that at any percentage moisture level, an eightfold increase in the ratio of silica gel to ascorbic acid caused no further increase in the ascorbic acid loss above that observed at the lower silica gel level in the 3 weeks at 45° storage tests. This lack of concentration effect of silica gel suggests strongly that the degradation of ascorbic acid is not due to surface interaction. Further, when 60% vitamin E oil was adsorbed on the silica gel, no change in the stability of ascorbic acid was observed. Thus is due undoubtedly to the strongly hydrophilic nature of this adsorbent.

It is of interest to examine the data in Fig. 1 in terms of the losses of ascorbic acid found at equivalent weights of water in the mixtures, rather than at equivalent percentages of water. This type of plot is given in Fig. 2. The circumstance that the higher proportion of silica gel exerts a protective effect indicates that some binding of water by the silica gel is taking place and suggests that the ascorbic acid losses are related to the amount of unbound or free water in the various mixtures. If this is true, then the equivalence of the ascorbic acid losses at the high and low silica gel levels at any particular percentage of moisture would indicate an equivalent amount of free water in these two mixtures.

Assuming that the silica gel binds water as a fixed fraction of its own weight at any given percentage of water, calculation has been made of the fraction that must be bound at both the 80 and 640-mg. silica gel levels in order to yield equal weights of free water at these two levels. A typical calculation is given below for the 25% water level in both mixtures, the compositions of which are as follows: (*a*) 80 mg. silica gel, 300 mg. ascorbic acid, and 127 mg. water; (*b*) 640 mg. silica gel, 300 mg. ascorbic acid, and 313 mg. water. If X = bound water level (expressed as percent of silica gel weight) which will yield the same weight of free water for both mixtures, then in both cases the total water minus bound water = free water, and

$$127 - 80 \times \frac{X}{100} = 313 - 604 \times \frac{X}{100}$$

from which $X = 33.2$. Then milligrams of bound water are: (*a*) $80 \times 0.332 = 26.6$ and (*b*) $640 \times 0.332 = 212.6$, and milligrams of free water are: (*a*) $127 - 26.6 = 100.4$ and (*b*) $313 - 212.6 = 100.4$.

These percentages of bound water and the corresponding weights of free water at the various total water levels are listed in Table I, together with the respective losses of ascorbic acid taken from the curve in Fig. 1. Values at the lower moisture levels are not included in Table I since the magnitude of the ascorbic acid losses in this range of water content is not sharply defined, due to the difficulty of mixing the small quantities of water uniformly, the greater error inherent in the small differences in titrations before and after storage, and the possible effect in some cases of a moisture loss during storage.

Table I—Effect of Moisture Content on Stability of Ascorbic Acid in Presence of Silica Gel[a]

H_2O Added, %	H_2O Added, mg.		80 or 640 mg. S.G.		
	80 mg. S.G.	640 mg. S.G.	Bound H_2O S.G. wt., %	Free H_2O, mg.	Loss A. A., %
25.0	127	313	33.2	100.4	31.3
29.4	159	392	41.6	125.7	39.7
33.3	190	470	50.0	150.0	47.0
36.9	222	550	58.6	175.0	54.6
40.0	254	627	66.6	200.7	62.3

[a] Ascorbic acid (A.A.), 300 mg. + 80 or 640 mg. silica gel (S.G.) + indicated percent H_2O—storage in closed tubes for 3 weeks at 45°.

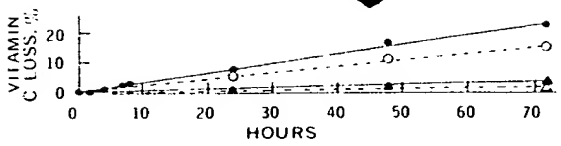


Figure 4 *Rate of loss of vitamin C in presence of silica gel; storage in closed tubes at 45° with 11.6% water. Key: △, 300 mg. ascorbic acid alone; ▲, 300 mg. ascorbic acid + 80 mg. silica gel; ○, 300 mg. sodium ascorbate alone; ●, 300 mg. sodium ascorbate + 80 mg. silica gel.*

The excellent correlation between weights of free water in the mixtures and losses of ascorbic acid is shown by the plot of Fig. 3. The losses of ascorbic acid are directly proportional to the calculated amounts of free water in the mixtures. For the sake of comparison, the data on the ascorbic acid plus water mixtures without silica gel are plotted in Fig. 3. The slope of the latter line is smaller than that of the ascorbic acid + silica gel + water mixtures. Again, in view of the fact that equal losses are found with a given weight of ascorbic acid over an eightfold range of silica gel plus bound water weights, it appears highly unlikely that surface reaction is a significant factor responsible for the higher losses of ascorbic acid in the presence of silica gel. It is believed more likely that trace metals such as iron and copper, which are present in silica gel to the extent of 110 and 1 p.p.m., respectively, are dissolved by the water and exert their well-known catalytic effect on ascorbic acid decomposition in solution (5, 6). Trace metals in the ascorbic acid (less than 10 p.p.m.) are present to the same extent in the tubes with or without silica gel. This mode of decomposition of ascorbic acid is in contrast to that reported by Carstensen *et al.* (7) for thiamine in solid dosage forms, where losses occur in an adsorbed surface monolayer of thiamine dissolved in water.

Rate of Loss of Ascorbic Acid and Sodium Ascorbate at 45° with 11.6% Water—These data are shown in Fig. 4. The loss of ascorbic acid alone in 3 days at 45° is only about 1%; in the mixture with silica gel the loss in this period is 3.6%. This small effect of silica gel in the 3-day test shows the same trend as described previously for the 3-week test.

The losses of sodium ascorbate, both with and without silica gel, are considerably higher than those found with ascorbic acid. This is to be expected in view of the fact that aerobic oxidation of ascorbic acid in the presence of metallic catalysts proceeds more slowly in acid solution than in neutral solution (8).

Effect of Other Tablet Excipients—The losses of ascorbate in the presence of the various excipients at 11.6% water are listed in Table II. Like silica gel, all these other excipients also increase the loss of ascorbate. The magnitude of the effect is undoubtedly dependent on the factors discussed above, including pH, water-binding capacity of the adjuvant, and trace metal content.

Granulation and Tablet Trials—Table III shows the excellent recoveries of ascorbic acid in dried granulations properly formulated with high levels of vitamin E in the form of silica gel adsorbates. Table IV similarly shows the excellent stability of sodium ascorbate in two dried granulations.

Finished tablets prepared by suitable techniques also show good stability of vitamin C in the presence of silica gel. This is demonstrated by the data in Table V on ascorbic acid stability in tablets prepared with 33 and 60% adsorbates of *d,l*-α-tocopheryl acetate on silica gel.

It has long been known that the sensitivity of vitamin C to oxidation in the presence of moisture is a factor that must be considered in the preparation of multivitamin tablets by wet granulation procedures. This is true whether or not silica gel is present in the granulation. In granulations containing appreciable quantities of silica gel and/or excipients, which are also potential contributors to vitamin C breakdown, it is possible to obtain excellent recovery of either ascorbic acid or sodium ascorbate by suitable wet granulation procedures. However, it is essential that the moisture level be held to the minimum level for effective granulation and that drying be carried out promptly and efficiently.

Table II—Influence of Various Excipients Plus Water on Stability of Sodium Ascorbate[a]

Excipient	Loss of Ascorbate, %
None	14.5
Cornstarch	16.4
Dicalcium phosphate anhydrous	17.7
Dicalcium phosphate dihydrate (milled)	19.0
Avicel	18.3
Silica gel	22.0
Tricalcium phosphate	25.3

[a] Excipient 80 mg. + 300 mg. sodium ascorbate + 11.6% water; storage for 3 days at 45°.

Table III—Recovery of Ascorbic Acid in Granulation and Tablets[a]

Lot No.	Vitamin E,[b] %	Excipient Added	Recovery Theoretical, % Granulation	Tablets
73-69/1	33	Dicalcium phosphate dihydrate	98	98
73-69/3	60	Same	100	100
73-69/5	60	Dicalcium phosphate anhydrous	100	100
73-69/6	60	Tricalcium phosphate	100	99

[a] Theoretical content: vitamin C = 77 mg.; vitamin E = 30 mg. [b] Adsorbate of *d,l*-α-tocopheryl acetate on silica gel.

Table IV Stability of Na Ascorbate in Granulation with Vitamin E Containing Silica Gel

Storage Test	Na Ascorbate, Theoretical, % Lot 811-58	Lot 811-59
Initial	97	98
3 weeks at 25°	98	96
3 weeks at 45°	96	96
3 weeks at 55°	96	96

Table V—Stability of Ascorbic Acid in Coated Multivitamin Plus Iron Tablets

Storage Test	Ascorbic Acid, Theoretical, % Maintenance Formula[a]	Therapeutic Formula[b]
1 month at 55°	100	91
3 months at 45°	100	94
6 months at 37°	97	88
12 months at 25°	100	94

[a] Contains 30 mg. of *d,l*-α-tocopheryl acetate per tablet as a 33% adsorbate on silica gel. [b] Contains 30 mg. of *d,l*-α-tocopheryl acetate per tablet as a 60% adsorbate on silica gel; both formulas have a theoretical ascorbic acid content of 77 mg. per tablet.

Table VI—Physiological Availability of Ascorbic Acid from Tablets Containing Silica Gel—24-hr. Test

	Dose (about 450 mg.) Excreted, %				
			Multivitamin Tablets, Vitamin E, 30 mg.		
Subject	Standard Ascorbic Acid Dose 1	Dose 2	Lot No. 73-69/1 (33% E Adsorbate) Initial	3 mo./45°	Lot No. 73-69/3 (60% E Adsorbate) Initial
BM	45	28	33	32	25
MO	52	57	36	37	46
RG	24	34	41	36	51
JS	39	40	41	53	41
ED	38	36	43	46	39
Average Availability ±*SE*, %	39.3		38.3 99 ± 9	40.8 103 ± 14	40.4 104 ± 13

Table VII--Physiological Availability of Ascorbic Acid from Tablets Containing Silica Gel—6-hr. Test

Subject	Dose (about 450 mg.) Excreted, %: Standard Ascorbic Acid	Lot No. 73-69/1 (33% E Adsorbate) Initial	Lot No. 73-69/1 (33% E Adsorbate) 3 mo./45°	Lot No. 73-69/3 (60% E Adsorbate) Initial
BM	28	27	27	17
MO	35	25	27	28
RG	18	26	26	37
JS	23	33	28	29
ED	26	29	28	24
Average	26.0	28.0	27.2	26.8
Availability $\pm SE$, %		108 ± 13	105 ± 11	103 ± 17

Experiences with wet granulation formulations containing ascorbic acid or sodium ascorbate indicate that excellent stability can be achieved if the wet granulation is dried to about 10% moisture within a few hours and to the final, low moisture content within 24 hr.

Order of mixing of ingredients and especially the mode of addition of water can be important. The vitamin C stability may be influenced by the presence of adsorbents that bind water or soluble ingredients that serve as emulsifiers or influence the solubility or reactivity of vitamin C.

Availability Studies in Men—The results of bioavailability tests in men of ascorbic acid in tablets containing silica gel have been calculated on the basis of 24-hr. excretions of test doses. These results are summarized in Table VI. Both lots of tablets show complete bioavailability of the ascorbic acid, and this was not changed by storage for 3 months at 45°. In order to provide information on the question of whether or not silica gel reduces the rate of absorption of ascorbic acid *in vivo*, calculations of physiological availability also were made on the basis of urinary excretions in the first 6 hr. after dose. These data are given in Table VII. Again, the results show complete availability of ascorbic acid in all three tablet trials, indicating that the ascorbic acid is absorbed normally in the presence of silica gel.

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Interfacial Barriers in Interphase Transport II: Influence of Additives upon the Transport of Diethylphthalate Across the Hexadecane-Gelatin-Water Interface

ABDEL-HALIM GHANEM, W. I. HIGUCHI, and A. P. SIMONELLI

Abstract □ The authors recently described a novel method for investigating the effects of an interfacial barrier in interphase transport. The procedures, both theoretical and experimental, were applied to the study of the effects of an adsorbed gelatin at the hexadecane-water interface upon the transport of diethylphthalate between the two phases. The present paper describes the influences of surfactants, electrolyte type, and concentration upon the permeability coefficient for the interfacial barrier. Experiments were conducted as before, employing diethylphthalate as the solute. The transport data were analyzed by the physical model described earlier. The results showed that the two ionic surfactants, sodium lauryl sulfate and dodecylpyridinium chloride, markedly decreased (2 to 12 times) the interfacial barrier even at low concentration (0.001–0.10% in the stock emulsion). Furthermore, the analysis showed that neither the electrolyte type nor concentration influenced the permeability coefficients, although they significantly altered the interphase transport rates themselves by changing the partition coefficients. These findings are particularly interesting as they may represent types of nonspecific situations that give rise to important barriers in *in vivo* drug transport.

Keyphrases □ Transport, interphase—interfacial barriers □ Diethylphthalate transport—hexadecane-gelatin-water interface □ Electrolyte effect—diethylphthalate transport, hexadecane-gelatin-water interface □ Surfactant effect—diethylphthalate transport, hexadecane-gelatin-water interface □ Permeability coefficients, interfacial barriers—surfactant, electrolyte type, concentration effect.

Recent studies from these laboratories (1, 2) involving the use of a novel method for investigating interfacial barriers in interphase transport have shown that substances adsorbed at the oil-water interface may control the interphase transport rates of solutes. Gelatin adsorbed at the hexadecane-water interface has been shown (1) to give an interphase transport rate for diethylphthalate that is about 1×10^4 times slower than diffusion controlled. A significant reduction in the aqueous to lipid transport rate of cholesterol by an

Exhibit I

Chemical Stability of Pharmaceuticals

A Handbook for Pharmacists

Second Edition

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To the memory of Lloyd Kennon

CHAPTER 5

Oxidation and Photolysis

Oxidative and photochemical reactions are, for the most part, one-electron reactions as opposed to reactions discussed in Chapter 4, which are two-electron reactions. For the hydrolytic reactions in Chapter 4, a free pair of electrons on a heteroatom in one molecule, a nucleophilic center, attacked an electrophilic center on a second molecule, whereas oxidative and photochemical reactions proceed through free radical or free-radical-like reaction pathways.

Most drugs exist in a reduced form, so the presence of 20% oxygen in the atmosphere creates obvious potential stability problems for these molecules. That is, many molecules tend to be converted to a more oxidized state. Kinetically, however, there is a sufficient energy barrier to many such reactions (the energy of activation) that not all molecules are subject to measurable rates of spontaneous oxidation or autoxidation. The radiation from the sun and artificial light, particularly visible and ultraviolet light, is also ubiquitous, so that molecules capable of rearranging upon absorption of radiation energy must be protected.

Our overall mechanistic understanding of oxidative and photochemical reactions is poor. The reason for this will be understandable as this chapter proceeds. Simply stated, many oxidative and photochemical reactions involve very complex reaction pathways with multiple intermediates so that even though the stoichiometry of a reaction might be given by Eq. (5.1) the kinetic law is *not* as simple as Eq. (5.2).

$$RH + O_2 \longrightarrow ROOH \qquad (5.1)$$

$$\frac{d[ROOH]}{dt} = k[RH][O_2] \qquad (5.2)$$

Also, unlike two-electron reactions where catalysis is often limited to acid/base or nucleophilic catalysis, trace quantities of environmental agents can powerfully catalyze one-electron reactions. For example, trace contamination of metal ions can catalyze oxidative reactions by many orders of magnitude, and the presence of a photosensitizing agent can cause a molecule that in the absence of the photosensitizing agent is not photolabile to undergo an apparent photochemical reaction.

In this chapter we introduce, from a basic viewpoint, the kinetics and other factors affecting oxidative and photochemical reactions and describe how these reactions can be prevented or at least inhibited.

A. OXIDATION

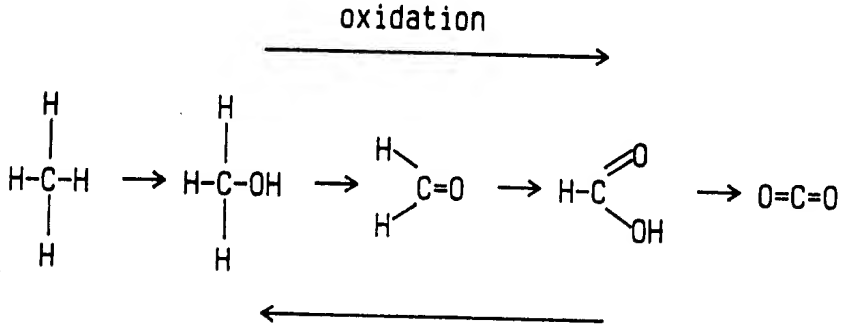
1. Nature of Oxidation

When one considers oxidation, it is important to realize that this reaction is a complementary one; its partner is reduction. One cannot happen without the other. Oxidation/reduction (redox) reactions involve the transfer of one or more oxygen or hydrogen atoms or the transfer of electrons. The classical, and familiar, inorganic redox system can be described by Eq. (5.3), where e^- represents an electron and n the

$$\text{reduced form} \rightleftharpoons \text{oxidized} + ne^- \qquad (5.3)$$

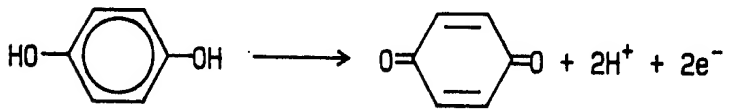
number of electrons. Thus redox reactions are electron-transfer processes, and this aspect must be considered if the basic process is to be understood.

In the case of organic compounds and especially the oxidation state of carbon, the oxidation state is determined by the number of bonds from carbon to oxygen. For example, the state of oxidation of one-carbon compounds increases as shown in Eq. (5.4). As stated earlier, the mechanism of this process is not as simple as suggested by the stoichiometry of the reaction.



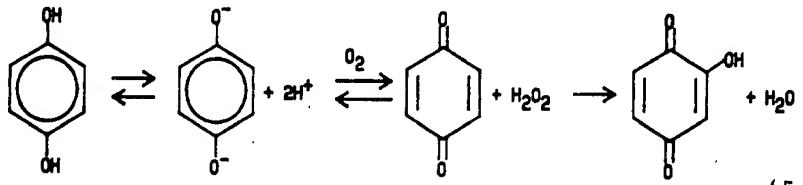
(5.4)

Also the simple redox system illustrated by Eq. (5.3) is made more complex by the medium in which the reaction occurs. For example, the oxidation of hydroquinone (1,4-dihydroxybenzene) to its quinone (*p*-benzoquinone) is often illustrated in the textbooks by Eq. (5.5).



hydroquinone quinone (5.5)

Yet in aqueous solution, free electrons, e^-, do not exist and the state of ionization of the hydroquinone is affected by the solution pH. Therefore in aqueous solution the oxidation of hydroquinones is more accurately described by Eq. (5.6).



(5.6)

As will be discussed later, the oxidation of hydroquinone and other phenols is even more complex than shown by Eq. (5.6) in that the product of the immediate oxidation, the quinone, can catalyze the oxidation

further. This process is called autocatalysis or product catalysis.

2. Kinetics of Oxidation

Oxidations that take place spontaneously under mild conditions are often called "autoxidation"; the majority of these are free-radical reactions. Free radicals are chemical species that possess an unpaired electron. Oxygen, in its ground state, is a diradical with the electronic configuration

Oxygen, therefore, would "like" to fill its outer electron shell to produce O_2^{2-}, the peroxy dianion. To do so, oxygen must accept two electrons from a donor molecule(s) and in so doing could in theory generate other free-radical molecules.

In most autoxidation reactions, even though oxygen is often involved, the initiation of the oxidation reaction does not involve molecular oxygen, that is, oxygen itself in its ground state does not really initiate oxidation reactions. Let us consider the general kinetic behavior of olefin autoxidation described by Eqs. (5.7)-(5.9)

$$R'' + R''\text{-}CH_2\text{-}CH{=}CH\text{-}R''' \xrightarrow{k_1} R''\text{-}\dot{C}H\text{-}CH{=}CH\text{-}R''' + R'H \quad (5.7)$$

$$R''\text{-}\dot{C}H\text{-}CH{=}CH\text{-}R''' + O_2 \xrightarrow{k_2} R''\text{-}\underset{\displaystyle O\text{-}O\cdot}{CH}\text{-}CH{=}CH\text{-}R''' \quad (5.8)$$

$$R''\text{-}\underset{\displaystyle O\text{-}O\cdot}{CH}\text{-}CH{=}CH\text{-}R''' + R''\text{-}CH_2\text{-}CH{=}CH\text{-}R''' \xrightarrow{k_3} R''\text{-}\dot{C}H\text{-}CH{=}CH\text{-}R''' + R''\text{-}\underset{\displaystyle O\text{-}OH}{CH}\text{-}CH{=}CH\text{-}R''' \quad (5.9)$$

a hydroperoxide

The reaction given by Eq. (5.7) represents the *initiation* reaction. Generally the species R'' is not

oxygen but some other peroxy radical present in the solution, trace quantities of metal ions such as ferrous or cupric ions, or radicals formed in the solution from the absorption of light (visible or ultraviolet).

The reactions given by Eqs. (5.8) and (5.9) represent *chain propagation* reactions, i.e., one radical produces one radical plus a hydroperoxide molecule. Radical species in solution, apart from reacting with oxygen or another unreacted molecule to produce another free radical, can also react with each other to produce stable or metastable products. This step is called a *chain termination* step and three examples are given in Eqs. (5.10)-(5.12).

$$\left.\begin{aligned} RO_2\cdot + RO_2\cdot &\xrightarrow{k_4} \\ RO_2\cdot + R\cdot &\xrightarrow{k_5} \\ R\cdot + R\cdot &\xrightarrow{k_6} \end{aligned}\right\} \text{stable products} \qquad (5.10)\text{-}(5.12)$$

Considering this reaction mechanism, and simplifying Eqs. (5.7)-(5.9) to Eqs. (5.13)-(5.15),

$$\text{initiator} \xrightarrow{k_1} R\cdot \qquad (5.13)$$

$$R\cdot + O_2 \xrightarrow{k_2} RO_2\cdot \qquad (5.14)$$

$$RO_2\cdot + RH \xrightarrow{k_3} ROOH + R\cdot \qquad (5.15)$$

assuming normal levels of oxygen, applying a steady-state assumption to the radical species $RO_2\cdot$ and $R\cdot$, letting $k_2[R\cdot][O_2] = k_3[RO_2\cdot][RH]$, and $k_4 = k_5 = k_6 = k_t$, it can be shown that the rate of hydroperoxide formation is given by Eq. (5.16) where r_i is the initiation rate.

$$\frac{d[ROOH]}{dt} = \left(\frac{r_i}{k_t}\right)^{1/2} k_3[RH]\,\frac{k_2[O_2]}{k_3[RH] + k_2[O_2]} \qquad (5.16)$$

It is obvious from this expression that the rate of hydroperoxide formation is proportional to the *square root* of the initiation rate, r_i. Also, if oxygen concentration is very high, $k_2[O_2] >> k_3[RH]$; Eq. (5.16) collapses to Eq. (5.17):

$$\frac{d[ROOH]}{dt} = \left(\frac{r_i}{k_t}\right)^{1/2} k_3[RH] \qquad (5.17)$$

The reaction will apparently be first order in starting material, RH. On the other hand, if $k_3[RH] >> k_2[O_2]$, then Eq. (5.18) is realized.

$$\frac{d[ROOH]}{dt} = \left(\frac{r_i}{k_t}\right)^{1/2} k_2[O_2] \qquad (5.18)$$

Under these conditions the reaction will be "pseudo zero order" with respect to RH, that is, [RH] does not appear in Eq. (5.18), and the reaction will be first order in $[O_2]$. If the term r_i actually involves RH or O_2, the order of the reaction with respect to RH and O_2 could be as high as 1.5.

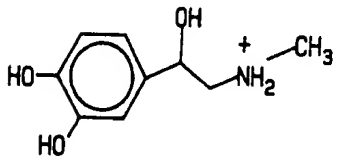
The mechanism defined by Eqs. (5.7)-(5.15) assumes that the species ROOH is stable and that the termination products are stable. In the case of olefins, the hydroperoxide can break down to produce volatile and nonvolatile products as well as multiple radicals.

$$\begin{array}{l} \text{O-OH} \\ \text{|} \\ \text{R"-CH-CH=CH-R"'} \end{array} \longrightarrow \text{R"-CHO} + \text{R"'-CH=CH}\cdot + \cdot\text{OH} \qquad (5.19)$$

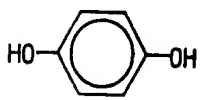
The rancidity of unsaturated cooking oils and oil-based paints is the result of this fragmentation of olefinic bonds to produce aldehydes, acids, and alcohols as well as multiple radicals. As can be seen in Eq. (5.19), one hydroperoxide molecule produces two radicals. If this reaction is favorable, not only do we have a chain propagation reaction, but chain branching reactions will be observed. If branching does occur, the oxidation kinetics and products become even more complex than those defined by the initiation, propagation, and termination sequence.

Qualitatively, the kinetics of oxidative free-radical reactions follow the pattern illustrated in Figure 5.1. A characteristic of many such reactions is a lag time or lag phase corresponding to the gradual buildup of radicals via the initiation step. If the radicals

at higher pHs. This suggests that it is the anionic form of the phenol that is most sensitive to oxidation. In the case of dihydroxybenzenes, such as epinephrine and hydroquinones, the rate of oxidation often exhibits apparent second-order dependency on

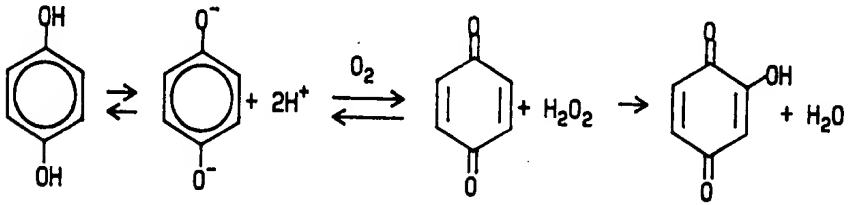


epinephrine



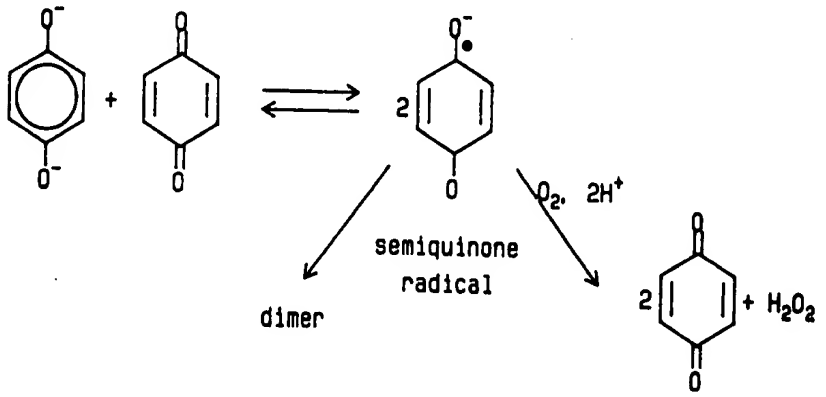
hydroquinone

hydroxide ion concentration. The oxidation rates of hydroquinone and alkyl-substituted hydroquinones have been extensively studied because of their use in the photographic industry (2). Their mechanism of oxidation appears to be



$HQ{\cdot}H_2$ HQ^{2-}

However, it was found that the kinetics of oxidation of hydroquinone seemed to be dependent on quinone concentration; that is, the quinone, the immediate product of the reaction, catalyzed the oxidation of the hydroquinone. This was explained by the following reaction, in which the quinone reacts with the dianion of the hydroquinone to form a very unstable semiquinone radical, which very rapidly and spontaneously reacts with oxygen to form two molecules of the quinone and hydrogen peroxide (H_2O_2), or dimerizes to a stable product (3).



In both of the above reaction schemes it is the dianion of the hydroquinone that appears to be the reactive species. The two pK_a's of hydroquinone are >9 and under pH conditions found in most formulations the fraction of hydroquinone present as its dianion is given by Eq. (5.29), where Ka_1 and Ka_2 are the first and second dissociation constants of hydroquinone.

$$f_{HQ^{2-}} = \frac{[HQ^{2-}]}{[HQ]_{TOTAL}} = \frac{Ka_1Ka_2}{[H^+]^2 + [H^+]Ka_1 + Ka_1Ka_2} \qquad (5.29)$$

With the conditions $[H^+] >> Ka_1$ and Ka_2, Eq. (5.29) becomes Eq. (5.30).

$$f_{HQ^{2-}} = \frac{Ka_1\ Ka_2}{[H^+]^2} = \frac{Ka_1\ Ka_2}{K_w^2}[OH^-]^2 \qquad (5.30)$$

Therefore, if the rate of oxidation of the hydroquinone is proportional to the hydroquinone dianion concentration, it can be seen that the rate of oxidation will be apparent second-order in hydroxide ion concentration. This is confirmed by the data in Fig. 5.2 for the oxidation of *m*-dimethylhydroquinone and hydroquinone in aqueous buffer solutions. Similar pH dependencies on the rate of oxidation of ascorbic acid (see curves 3 and 4 of Fig. 1 of the L-ascorbic acid monograph) and captopril (see Fig. 1 of the captopril monograph) have been observed. In each case the rate of oxidative decomposition under aerobic conditions is

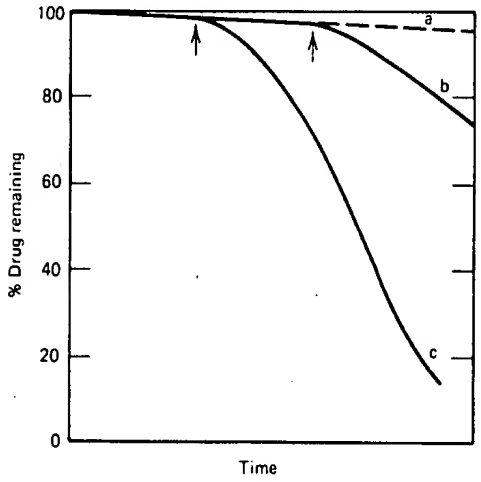


FIGURE 5.1. Illustration of percentage of drug remaining vs. time for an oxidative free-radical reaction: curve a initiation step only; curve b initiation plus propagation; and curve c initiation, propagation, and chain branching. Arrows indicate the lag times.

produced from the initiation step are stable then as soon as the catalytic species is consumed the reaction stops (curve a in Fig. 5.1). If the radicals produced from the initiator go into a propagation cycle, curve b results. The overall loss of drug will then often follow a first-order decay curve with respect to drug, depending on the oxygen dependency of the reaction. If chain branching occurs the overall loss of drug shows an acceleration phase (see curve c) with maximum acceleration occurring at ~50% drug remaining.

The reaction kinetics defined by Eqs. (5.7)-(5.19) were for a reaction in which the reactant RH was not capable of ionization. For the oxidation of drugs or pharmaceutical additives, the kinetics are further complicated when the state of ionization of the molecule is affected by solution pH. In the oxidation of sodium sulfite (or bisulfite) a first approximation of the oxidation mechanism is given by Eqs. (5.20)-(5.27),

$$SO_3^{2-} + M^+ \rightleftharpoons SO_3^{-}\cdot + M\cdot \qquad (5.20)$$

$$SO_3^{-}\cdot + O_2 \rightleftharpoons SO_5^{-}\cdot \qquad (5.21)$$

$$SO_5^{-}\cdot + HSO_3^- \rightleftharpoons HSO_5^- + SO_3^{-}\cdot \quad (pH \leq 7) \qquad (5.22)$$

$$SO_5^{-}\cdot + SO_3^{2-} \rightleftharpoons SO_5^{2-} + SO_3^{-}\cdot \quad (pH \leq 7) \qquad (5.23)$$

$$SO_3^{2-} + HSO_5^- \rightleftharpoons HSO_4^- + SO_4^{2-} \quad (pH \leq 7) \qquad (5.24)$$

$$SO_3^{2-} + SO_5^{2-} \rightleftharpoons 2SO_4^{2-} \quad (pH \leq 7) \qquad (5.25)$$

$$SO_3^{-}\cdot + SO_5^{-}\cdot \rightleftharpoons S_2O_6^{2-} + O_2 \qquad (5.26)$$

$$SO_5^{-}\cdot + \text{inhibitor} \rightleftharpoons \text{nonreactive products} \qquad (5.27)$$

where Eq. (5.20) is the initiation step, Eqs. (5.21)-(5.23) are propagation steps, Eqs. (5.24) and (5.25) oxidation steps leading to the ultimate oxidation product, SO_4^{2-}, and Eqs. (5.26) and (5.27) termination steps. M^+ is a metal ion catalyst. The overall kinetics of sulfate formation or sulfite loss is very complicated, although it has been shown that at pHs < 8 the proportionality given by Eq. (5.28) is observed (1).

$$\frac{d[SO_4^{2-}]}{dt} \alpha\ [M^+][SO_3^{2-}][HSO_3^-] \qquad (5.28)$$

Note that in this expression there is no oxygen concentration dependency, that the reaction proceeds faster at higher pHs, and that the reaction is sensitive to metal ion catalysis, especially by Fe^{3+}, Mn^{2+}, and Cu^{2+}. The pH dependency arises because the fractions of sulfite and bisulfite ions are pH dependent.

The kinetics and mechanism of oxidation of phenols and substituted *o*- and *p*-dihydroxybenzenes in aqueous solution are also very complex and pH dependent. In general terms the oxidation of such molecules is very sensitive to the presence of metal ions, oxygen concentration, and pH, with increasing rates of oxidation

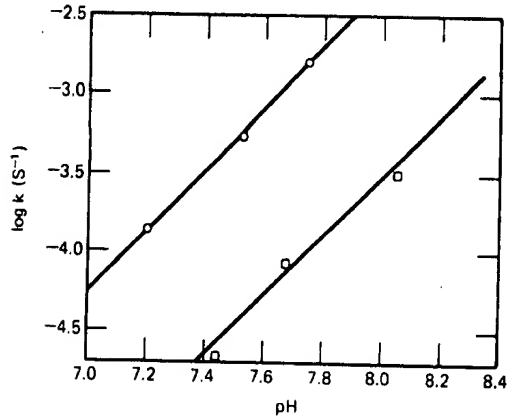


FIGURE 5.2. pH-rate profiles for the oxidation of hydroquinone (⊡) and *m*-dimethylhydroquinone (⊙) at 25°C. The slopes of the lines are 1.96 and 1.98, respectively. Both systems were studied in phosphate buffer.

proportional to the fraction of the drug in the anionic form, namely the ascorbate anion or the thiolate (RS^-) in the case of captopril.

Interpretation of temperature effects on oxidative reactions is made difficult by the multiple steps in many of the reactions and because oxygen solubility in water (and other solvents) is temperature dependent. Since each reaction in a complex scheme will have its own activation energy, it is possible that as the temperature is changed a different reaction will become rate determining. Theoretically, under such circumstances, the adherence of the reaction-rate/temperature relationship to the Arrhenius equation will break down. Practically, however, over a limited temperature range, Arrhenius behavior may be observed, but the activation energy is very much an "apparent" activation energy for which the reaction conditions must be clearly stated. Included in this "apparent" activation energy is the temperature dependence of the oxygen solubility. Table 5.1 gives the O_2 content of water at various temperatures if the water is saturated by air or by pure oxygen. As can be seen for the air data, a 20°C change in temperature (5 $\rightarrow$ 25°C) results in a 40% decrease in oxygen concentration. If

TABLE 5.1. Oxygen Content of Water Under Air and Pure Oxygen at Atmospheric Pressure and Various Temperatures.

Temperature (°C)	Millimoles of O_2 from air per mL of H_2O[a,b]	Millimoles of O_2 from pure O_2 per mL of H_2O[b,c]
0	--	2.18×10^{-3}
5	0.386×10^{-3}	--
10	0.34×10^{-3}	--
15	0.304×10^{-3}	--
20	0.267×10^{-3}	--
25	0.232×10^{-3}	1.29×10^{-3}
50	--	9.28×10^{-4}
100	--	7.51×10^{-4}

[a]From Reference 4.
[b]Calculated from cc of O_2 in H_2O and the expression $PV = nRT$.
[c]From Reference 5.

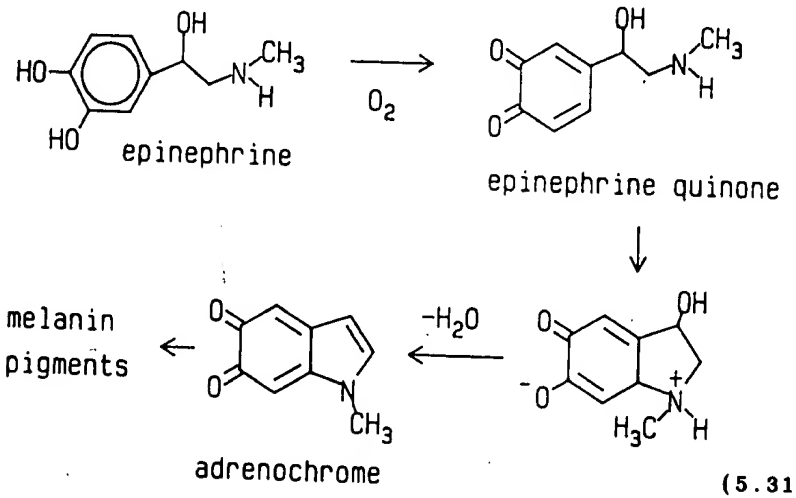
the rate of reaction under study is first order in oxygen concentration, then a ninefold increase in rate ($Q_{10} = 3$) due to the direct effect of temperature on the rate-controlling step will show up experimentally as only a 5.5-fold increase in rate owing to the concomitant change in oxygen concentration.

3. Oxidative Pathways of Pharmaceutical Interest

A few selected oxidative reactions of pharmaceutical interest are illustrated here; the stability monographs include other examples. Many drug compounds have been reported to be subject to autoxidation, including adriamycin hydrochloride, amphotericin B, apomorphine, ascorbic acid, capropril, chlorpromazine and other phenothiazine derivatives, cyanocobalamin, cysteine, epinephrine, ergometrine, hydrocortisone, isoamyl nitrite, isoproterenol, kanamycin, 6-mercaptopurine, morphine, neomycin, norepinephrine, novobiocin, *p*-aminobenzoic acid, paraldehyde, penicillin, phenylephrine, physostigmine, prednisolone, prednisone,

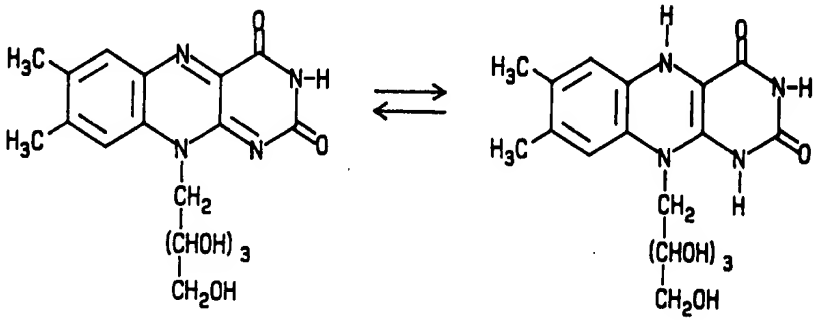
procaine, resorcinol, riboflavin, streptomycin and dihydrostreptomycin, sulfadiazine, terpenes, the tetracyclines, thiamine, and vitamins A, D, and E. The unwanted conversion of fats, oils, flavors, and perfumes to a rancid state is due to oxidation of these unsaturated molecules. The double bonds are oxidized to form hydroperoxides, as demonstrated earlier, which then produce aldehydes; the latter cause the offensive odors and unpleasant flavors. Vitamin A (see monograph) and amphotericin B (see monograph) are two drug molecules with extended conjugated double bonds that are very susceptible to oxidative breakdown.

Epinephrine forms colored products on oxidation [Eq. (5.31)].



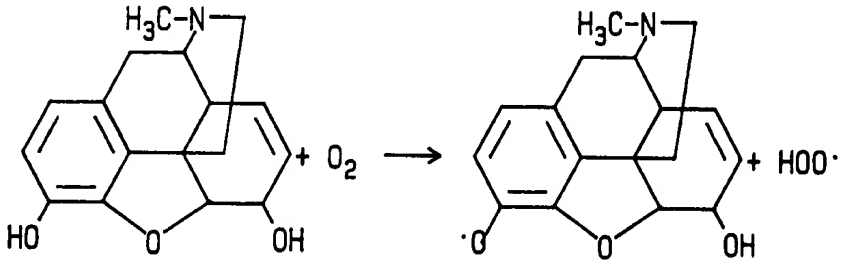
(5.31)

The activity of riboflavin is contingent on its ability to take part in this redox equilibrium:



(5.32)

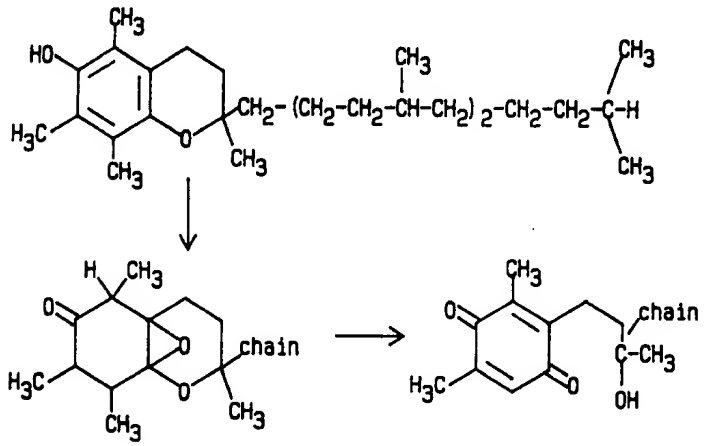
Morphine dimerizes when oxidized. Equation (5.33) shows the first step.



(5.33)

The formed morphine free radical couples with a morphine molecule (at the free position ortho to the phenolic oxygen) to give the dimer (bimorphine or pseudomorphine). Hydrogen peroxide is also produced and can cause additional oxidation to the N-oxide.

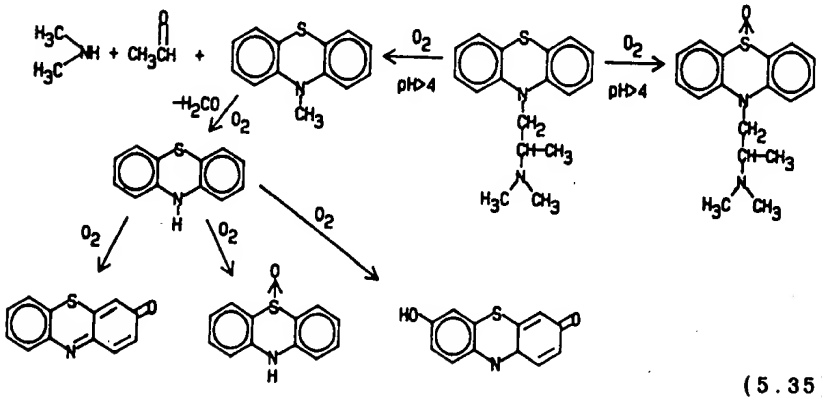
Vitamin E can form an epoxide, which then produces a quinone: vitamin E (α-tocopherol):



vitamin E epoxide α-tocopherylquinone

(5.34)

The phenothiazines readily oxidize, producing a multitude of products. The degradation mechanism of promethazine and the influence of pH, metals, chelating agents, and antioxidants, have been extensively studied (6,7). The following reactions have been proposed and their products isolated.



(5.35)

Sulfide and sulfhydryl-containing molecules are particularly vulnerable to oxidation. Captopril (see

monograph) is an example of a recently developed sulfhydryl drug with oxidative stability problems. Sulfhydryl-containing molecules generally oxidize to their corresponding disulfides, Eq. (5.36).

$$R\text{-}SH \xrightarrow{\frac{1}{2}O_2} R\text{-}S\text{-}S\text{-}R + H_2O \qquad (5.36)$$

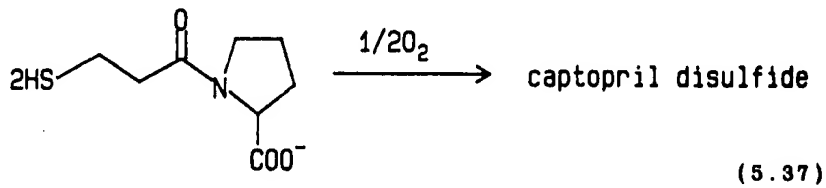
4. Inhibition of Oxidation

Exclusion of Oxygen

If a molecule requires the presence of oxygen to degrade why not exclude oxygen from the formulation? For parenteral drugs this can be achieved by packaging the drug in glass ampuls that are heat sealed under an inert atmosphere. For tablets, capsules, and so on, packaging of the formulation in a hermetic strip may be useful in preventing the oxidation.

Quite often manufacturers would like to formulate a drug for parenteral administration in a multidose vial, which is sealed with a rubber stopper. It is very difficult to formulate very oxygen-sensitive drugs in such multidose vials because rubber is reasonably permeable to oxygen and both synthetic and natural rubber tend to release additives capable of catalyzing oxidative reactions. Oxidatively unstable drugs formulated in multidose vials require more than just oxygen exclusion during sealing to prevent oxidation from occurring.

The importance of deoxygenating the water and the headspace atmosphere in an ampul prior to sealing can be seen in the following calculation using captopril as an example. The stoichiometry of the oxidative breakdown of captopril is given by Eq. (5.37), showing that 2 mol of captopril are lost for each half-mole of oxygen consumed.



(5.37)

A 2 mg/2 mL injection of captopril, in a 2-mL ampul, has a headspace of ~1 mL. The 2 mg of captopril is equivalent to 9.2×10^{-3} mmol of captopril. At 25°C there are 0.23×10^{-3} mmol of dissolved oxygen in each mL of water for a total of 0.46×10^{-3} mmol of oxygen. The 1 mL of headspace, assuming that it is air, contains 8.6×10^{-3} mmol of oxygen. Based on the stoichiometry of Eq. (5.37) it can be seen that the 9.06×10^{-3} mmol of oxygen in the system would be capable of degrading 3.62×10^{-2} mmol of captopril, that is, there is more than enough oxygen present in this formulation to completely degrade the captopril. If the oxygen in the headspace is removed by flushing with an inert gas, the 0.46×10^{-3} mmol of oxygen in the water are still capable of degrading 1.84×10^{-3} mmol of captopril, or 20% of the formulation. By performing such calculations it is possible to predict how thorough the exclusion of oxygen from the system must be in order to prevent the oxidative breakdown of sensitive drugs.

Alteration of Solution pH

As has already been discussed, the oxidation of many drugs is pH sensitive. Acidic drugs such as ascorbic acid, phenols, and sulfhydryl compounds all degrade more rapidly in neutral to alkaline pH conditions. For such drugs the pH range 3 to 4 is generally found most useful in minimizing oxidation. Obviously, this pH range would not be useful for acidic drugs that have limited aqueous solubility at low pH values. Amine drugs such as the phenothiazines appear to be most stable in their protonated forms, that is, also at low pH values.

Protection from Light

Oxidative breakdown of drugs generally proceeds through the sequence of initiation, propagation (and maybe chain branching), and termination. As mentioned earlier, a triggering force that may promote oxidation is "light" (namely, certain components of the electromagnetic spectrum). Not all photolytic reactions are oxidative in nature and not all oxidative reactions require light as either an initiator or as an integral component of the propagation steps. If, however, light does initiate or promote an oxidative breakdown of a drug, the exclusion of the particular wavelength range of light responsible for the catalysis will often suppress the oxidation. This can be achieved by the total exclusion of light using an opaque container or the use of pigmented glass capable of excluding the damaging wavelengths. This will be discussed further in the section of this chapter on photolysis.

Use of Chelating Agents and Antioxidants

Oxidation reactions can be inhibited by agents that are

(a) chelating agents for metal ion initiators of free-radical oxidation reactions;
(b) reducing agents, that is, substances that can reduce an oxidized drug;
(c) preferentially oxidized compounds, that is, agents that are more readily oxidized than the agents they are to protect; or
(d) chain terminators, that is, agents capable of reacting with radicals in solutions to produce a new species, a chain terminator radical, which does not reenter the radical propagation cycle. The new radical may be intrinsically stable or may dimerize to form an inert molecule.

Compounds in all four categories are often classified as antioxidants (or antoxidants). It is probably more accurate to classify compounds in categories (b)-(d) as antioxidants and chelating agents as synergists. These categories are now treated in more detail.

(a) Chelating Agents. Oxidative reactions are often initiated by metal ions such as Fe^{3+}, Cu^{2+}, Co^{3+}, Ni^{2+}, Mn^{3+}. These metal ions act as initiators because in their oxidation states they are capable of acting as radicals. For example, Cu^{2+} has 27 electrons and requires one more electron to complete the electron pair.

Metal ions catalyze oxidative reactions in a number of ways. They can react directly with oxygen to produce an oxygen radical, which can then initiate an autoxidation. The metal ion can form a complex with oxygen and subsequently form a peroxy radical. The

metal ion can react with the drug itself to form a radical, as illustrated by Eq. (5.38), which is then able to enter into a propagation cycle [see Eqs. (5.7) and (5.13)].

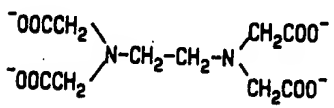
$$M^{n+} + RH \longrightarrow M^{(n-1)+} + H^+ + R\cdot \qquad (5.38)$$

The metal ion can also react with a hydroperoxide in the formulation to catalyze the breakdown, given by Eq. (5.39).

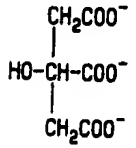
$$M^{n+} + R'OOH \longrightarrow M^{(n-1)+} + H^+ + R'O_2\cdot \qquad (5.39)$$

R'OOH could be a hydroperoxide of the drug itself or of some other component of the formulation.

Chelating agents act in an antioxidant capacity by binding metal ions, thus removing them, thermodynamically speaking, from solution. The most effective chelating agents used pharmaceutically are ethylenediaminetetraacetic acid (EDTA), citric acid, many of the amino acids, phosphoric acid (weak), and tartaric acid. EDTA and citric acid are the two most useful agents. Their metal-binding capacity is dependent on their state of ionization, both being most effective when their carboxylic acid groups are fully ionized. Thus, they lose their chelating capacity at low pH.



EDTA tetraanion (edetate)



citric acid trianion

Just because an agent is able to chelate metal ions does not mean that it will reduce the effectiveness of the metal ion to act as a catalyst. There are circumstances in which the metal ion may bind to some functional groups and in this bound capacity actually may be a better catalyst than in the unbound state. The chelating agents mentioned above, however, generally act to lower the catalytic activity of the metal ions towards radical chain reactions.

(b) Reducing Agents. This approach is generally not used as a means of preventing oxidation. Sodium thiosulfate and ascorbic acid are two reducing agents that have been used in this capacity.

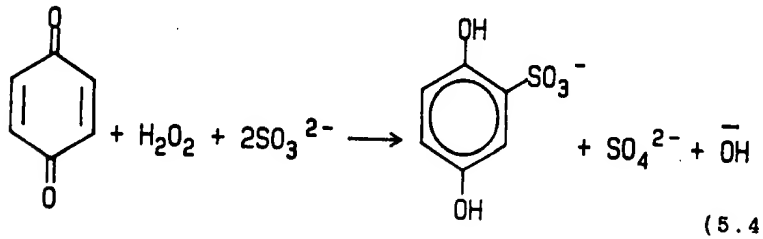
(c) Preferentially Oxidized Compounds. These are compounds that are more readily oxidized than the agents they are to protect. Essentially these agents act as oxygen scavengers. Two good pharmaceutical examples of oxygen scavengers are sodium bisulfite (and sulfite) and ascorbic acid. Sodium sulfite reacts with oxygen according to Eq. (5.40).

$$SO_3^{2-} + \tfrac{1}{2}O_2 \longrightarrow SO_4^{2-} \qquad (5.40)$$

It is possible to calculate the approximate amount of antioxidant needed to use up all the oxygen in an ampul, for example, by calculating the amount of oxygen dissolved in the water and the headspace of the ampul. In this example, 2 mol of sodium sulfite equivalents (as sodium bisulfite) would be needed to consume each mole of oxygen.

The sulfites are very commonly used, and a word of caution is in order. In the process of acting as antioxidants, sulfites yield acid sulfates, which cause a drop in pH. Also, sulfites can readily form inactive addition compounds, as with epinephrine (see monograph). They react with compounds such as alkenes, alkyl halides, and aromatic nitro and carbonyl compounds. Sometimes, as with thiamine, they may cleave molecules.

Although the nucleophilicity of the sulfite ions can be a disadvantage, there are circumstances where this nucleophilicity is an advantage. For example, in the oxidation of hydroquinone, it appears that the immediate product of the oxidation, its corresponding quinone, can act as a catalyst for further oxidation. Sodium sulfite (or bisulfite) can react with the quinone and hydrogen peroxide to form hydroquinone monosulfonate, and sulfate as described by Eq. (5.41).

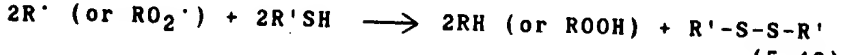


(5.41)

It appears that thiols can also act as antioxidants in a similar manner. All the major water-soluble antioxidants, namely acetone sodium bisulfite, ascorbic acid, cysteine hydrochloride, isoascorbic acid, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, sodium sulfite, thioglycerol, thioglycolic acid, and thiosorbitol, act as oxygen scavengers, although the thiol (sulfhydryl) group-containing antioxidants can also act as chain terminators (category d). Few if any of the lipid-soluble antioxidants act as true oxygen scavengers except for, perhaps, ascorbityl palmitate.

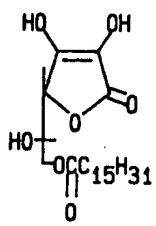
(d) Chain Terminators. Referring to the earlier discussions on the oxidation of olefins [Eqs. (5.7)-(5.15)], bisulfite [Eqs. (5.20)-(5.27)], and phenols, all of these reactions proceed through a radical mechanism. Therefore any substance that can donate a hydrogen radical while itself forming a radical that is stable and incapable of continuing the propagation chain cycle could act as an antioxidant. Such antioxidants act by being acceptors. They are also called chain terminators.

The major water-soluble antioxidants that can act as chain terminators are the thiol species cysteine, thioglycerol, thioglycolic acid, and thiosorbitol. These act by the mechanism described by Eq. (5.42).

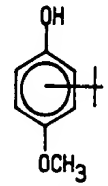


(5.42)

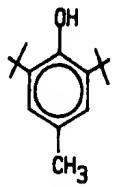
Essentially all the lipid-soluble antioxidants act as chain terminators. The major examples are



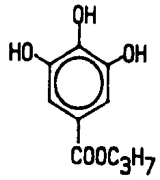
ascorbityl palmitate



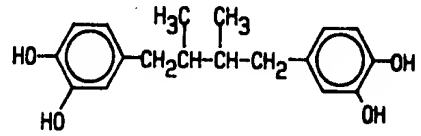
butylated hydroxyanisole (BHA)



butylated hydroxytoluene (BHT)



propyl gallate



nordihydroguaiaretic acid (NDGA)

as well as hydroquinone (see earlier structure), octyl and dodecyl gallates, α-tocopherol (see earlier structure), and phenyl α-naphthylamine.

To enhance the effectiveness of the antioxidant approach, it is sometimes useful to use more than one antioxidant. It has been found that a combination of two antioxidants, along with a chelating agent to complex metals (thus inhibiting their catalysis of the autoxidation), often works well. This enhanced effectiveness is often referred to as synergism.

The best studied examples of synergism are mixtures of a chelating agent with chain terminators. Figure 5.3 is a plot of peroxide formation with Oil of Terebinth (containing unsaturated hydrocarbons) as a function of time in the presence of various chelating agents (EDTA and citric acid), and various chain terminators (BHA, propylgallate, and NDGA). The superior antioxidant action of the mixture of chelating agent with a chain terminator is readily obvious.

Another good example of synergism is seen with the use of mixtures of hindered and unhindered phenols, for example, the use of BHT and BHA in many food and pharmaceutical products. BHA is a relatively unhindered phenol, whereas in BHT the phenolic hydroxy group is ortho to two large *t*-butyl groups. The synergism seen with this mixture occurs because the unhindered phenol (AH) readily reacts with radicals in

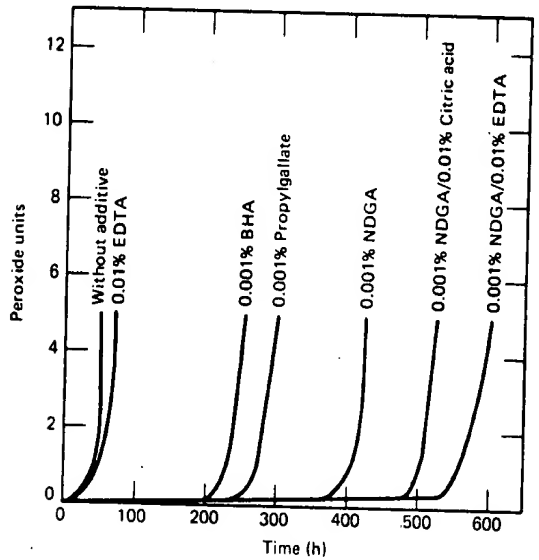


FIGURE 5.3. The effect of various antioxidants, chelating agents, and antioxidant/chelating agent mixtures on the formation of peroxides in Oil of Terebinth. [Data taken from L. Fryklof (8).]

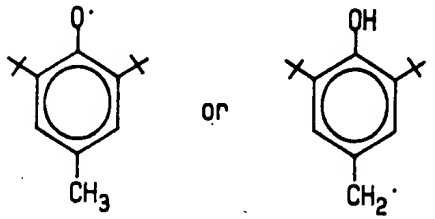
solution, Eq. (5.43), to produce a new radical, A·.

$$R\cdot \text{ (or } ROO\cdot) + AH \rightleftharpoons RH \text{ (or } ROOH) + A\cdot \qquad (5.43)$$

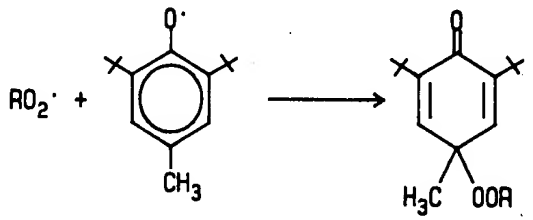
This radical could theoretically take part in further propagation steps. However, before a buildup of A· becomes a problem, it reacts with the less reactive hindered phenol, BH, to product the B· radical via Eq. (5.44).

$$A\cdot + BH \rightleftharpoons B\cdot + AH \qquad (5.44)$$

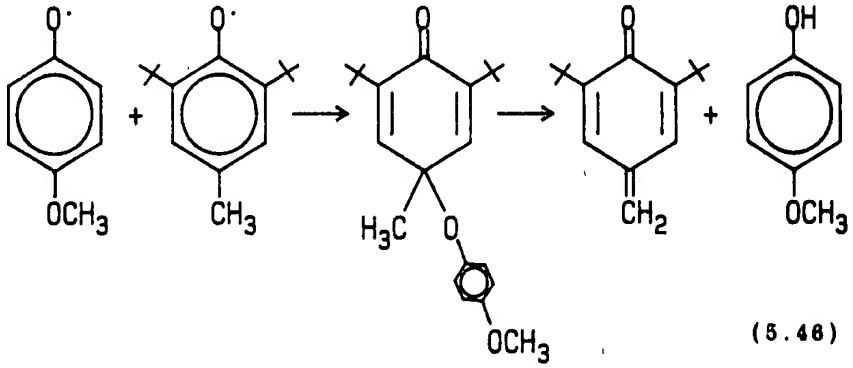
The radical B· is very stable per se and because of the hindered nature of the hydroxy group does not undergo further reaction. In the case of BHT the structure of B· has been postulated to be



There is evidence that reactions defined by Eqs. (5.45) and (5.46) also occur.



(5.45)



(5.46)

B. PHOTOLYSIS

1. Energetics of Photolysis

Photolysis is a consequence of the absorption of "light" or radiation energy, allowing for quantum restrictions, by a molecule A, to produce an unstable excited-state species, A*, Eq. (5.47). The absorbed

energy can be lost either by a radiative mechanism in which the energy is given off in the form of fluorescence or phosphorescence, Eq. (5.48), or by a radiationless mechanism, Eqs. (5.49)-(5.51). The radiationless mechanism can be physical or chemical in nature. The physical decay results in the loss of energy as heat, Eq. (5.49), or by collision with other molecules (quenching), Eq. (5.50). The net effect of the chemical decay is that sufficient energy is concentrated in some bond that the molecule chemically decomposes (or rearranges) into a new species, Eq. (5.51). This whole process can be defined by Eqs. (5.47)-(5.51). Photolysis is succinctly represented by Eqs. (5.47) to (5.51).

$$A \xrightarrow{h\nu} A^* \qquad (5.47)$$

$$A^* \xrightarrow{k_1} A + h\nu' \qquad (5.48)$$

$$A^* \xrightarrow{k_2} A + \text{heat} \qquad (5.49)$$

$$A^* + A \xrightarrow{k_3} 2A \qquad (5.50)$$

$$A^* \xrightarrow{k_4} \text{product(s)} \qquad (5.51)$$

According to the Stark-Einstein law the absorption of one quantum of radiation results in the formation of one photoexcited molecule. Since the photoexcited molecule may take part in several photochemical processes [Eqs. (5.48)-(5.51)], a quantum yield, ϕ, for any one of these processes is defined by

$$\phi = \frac{\text{number of molecules undergoing the process}}{\text{number of quanta absorbed}}$$

For a pure photochemical reaction the quantum yield has a value in the range 0-1. However, if A* is a radical or pseudoradical that can take part in an oxidative free-radical chain reaction, so that the absorption of energy simply initiates the reaction, then each quantum of energy may result in many molecules decomposing, in which case ϕ may appear to be greater than 1.

The energy per quantum of electromagnetic radiation is given by Eq. (5.52):

$$\text{energy} = h\nu \qquad (5.52)$$

where h = Planck's constant (6.625×10^{-27} erg-s), and ν is the frequency of the radiation in Hz (s^{-1}). Thus the shorter the wavelength (λ) or the higher the frequency (ν), the greater is the energy absorbed, since $\nu = c/\lambda$, where c is the velocity of light.

The energies of interest in the present context are associated with these portions of the spectrum (in order of decreasing energy and with wavelengths in nanometers following in parentheses): ultraviolet (UV) (50 to 400), visible (400 to 750), and infrared (IR) (750 to 10,000). An *approximate* visualization of the effects of the energies involved here is that the far and central portions of the infrared are sufficiently energetic to stimulate molecular translation and rotation; the molecular kinetic energy is increased, and rotational modes of motion are excited. The energy imparted from the near-IR to the edge of the visible region excites vibrational motions; that is, changes in bond lengths and angles; energy requirements lie in the range 1000 to 36,000 cal/mol. The wavelengths become shorter as we pass through the visible and UV portions of the spectrum, and the energetic effects on the molecules and their constituent atoms are much more marked. Electronic transitions and cleavage of chemical bonds may occur. The energies range from about 36,000 to 72,000 cal/mol (visible) to 72,000 to 286,000 cal/mol (UV). Clearly we are now dealing with energies capable of causing chemical reactions, and we anticipate that the greatest stability problems will arise from light of the shorter wavelengths, in particular from the short wavelength visible and the UV.

2. Kinetics of Photolysis

The simplest kinetic interpretation of photolysis is that given by Eqs. (5.47)-(5.51). In dilute solutions of drug A, the overall loss of drug, or rate of product formation, follows approximate first-order kinetics, whereas in more concentrated solutions the rate can approach pseudo-zero-order kinetics. The change in reaction order results because the reaction becomes

limited by the number of incident quanta of energy and because in concentrated solutions quenching of the excited molecules through Eq. (5.50) becomes more efficient.

In Eq. (5.50) it was assumed that A* collides with A to quench the excited state. Alternatively, A* could collide with any other molecule (quencher) or the container walls to help dissipate the energy. Also the reaction scheme defined by Eqs. (5.47)-(5.51) assumes that the initial energy absorption is by A, the molecule undergoing the decomposition. An alternative process is defined by Eqs. (5.53) and (5.54):

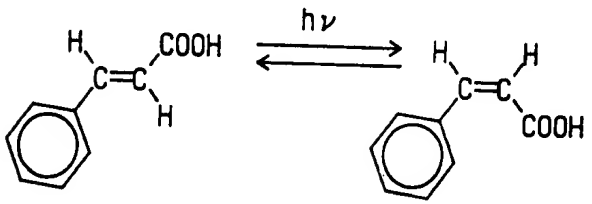
$$B \xrightarrow{h\nu} B^* \qquad (5.53)$$

$$B^* + A \longrightarrow A^* + B \qquad (5.54)$$

In this case the molecule B is called a photosensitizer, that is, A itself may not be capable of absorbing the radiation energy at frequency ν, but B can. B* then transfers the absorbed energy to A to produce A*.

3. Photolytic Reactions of Pharmaceutical Interest

Some reactions of pharmaceuticals in the presence of light are presented here. *trans*-Cinnamic acid isomerizes to its *cis* isomer in the presence of light, Eq. (5.55).



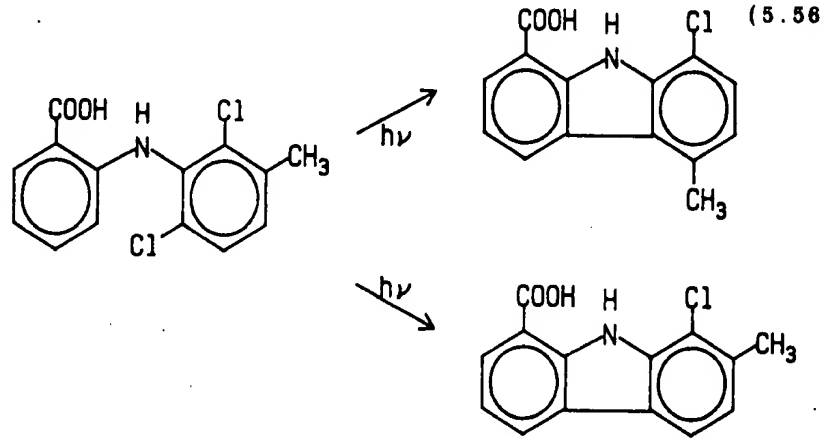
trans-cinnamic acid cis-cinnamic acid

(5.55)

These reactions occur in the presence of light because the carbon-carbon π electrons are excited into "antibonding" orbitals, which allow rotation. Note that the absorption of hν does not affect the equilibrium

constant for this reaction; it speeds the attainment of the equilibrium.

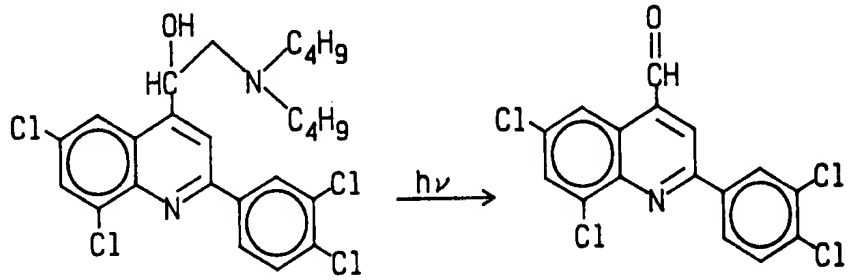
Meclofenamic acid undergoes dehydrohalogenation (9) into two products, Eq. (5.56) and (5.57).



(5.56)

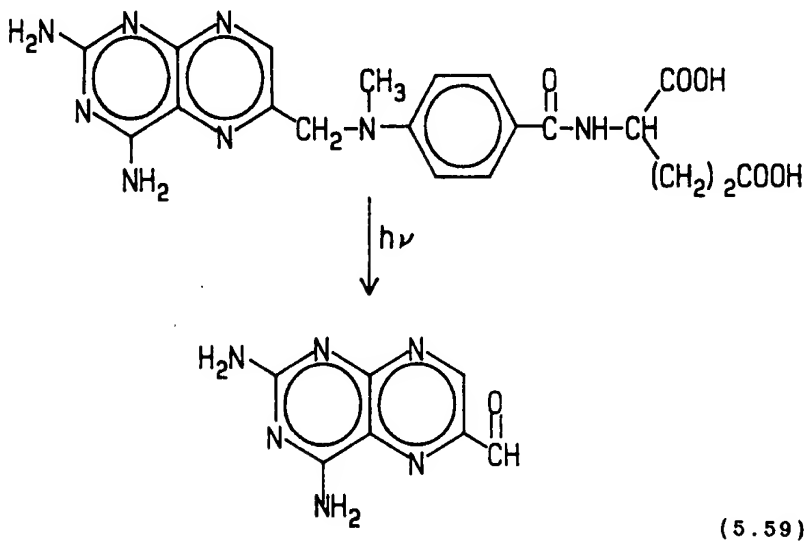
(5.57)

The experimental antimalarial WR 30090 (10) loses its side chain, Eq. (5.58).



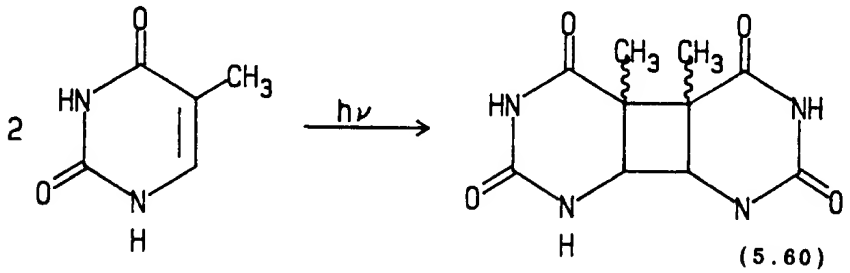
(5.58)

This reaction is very similar to the photolytic degradation of methotrexate, Eq. (5.59).



(5.59)

Dimerizations are sometimes catalyzed by light, Eq. (5.60).



(5.60)

Photoxidations are quite common with the phenothiazines. Solutions (5%) of two therapeutically useful phenothiazine salts, chlorpromazine hydrochloride and prochlorperazine ethanedisulfonate, were placed in a Warburg respirometer to permit measurement of oxygen uptake and were then exposed to a sunlamp. The solutions became colored shortly after the light was turned on, and they continued to darken. The data (11) are shown graphically in Figure 5.4. Other pharmaceutical products undergoing photolytic degradation are amphotericin B (see monograph), nitrofurantoin, furosemide, amyl nitrate, tetracycline(s), and ergotamine (see monograph).

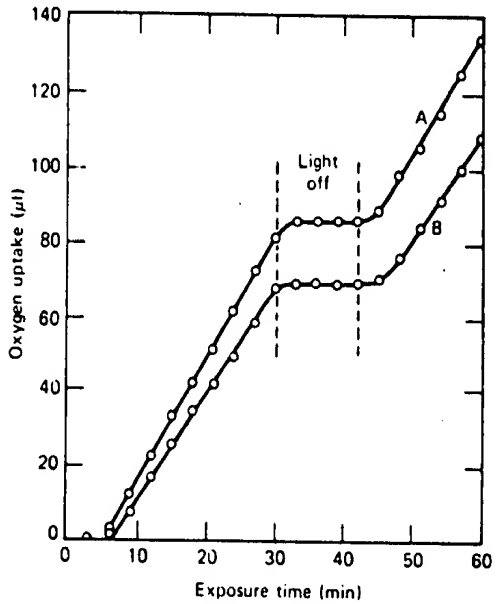


FIGURE 5.4. Plot of oxygen-uptake data for chlorpromazine hydrochloride (A) and prochlorperazine ethanedisulfonate (B) illustrating induction period, the linearity of the uptake with time, and the extreme light dependence of the oxidative degradation (11).

4. Prevention of Photolytic Reactions

Photolytic reactions are easily prevented by excluding light. This can be done by packaging the drug product in containers that are opaque, that is, all light is excluded, or that filter out all the light of those wavelengths that catalyze the reaction.

The light-transmission limits of glass and plastic containers are specified by the USP (12). These limits are given as the maximum percent transmission for any wavelength between 290 and 450 nm, for vessels of nominal capacities up to 50 mL. For containers larger than 50 mL, the limits for 50 mL apply. Light transmission for containers of type NP glass and for plastic containers for products intended for oral or topical use must not exceed 10%.

To understand the subject of light transmission through glass a little more thoroughly, note that a beam of light falling perpendicularly on a flat transparent surface loses some of its energy by reflection. If the beam impinges at an angle other than 90°, the loss is even greater. The transparent material then absorbs part of the energy entering it and transmits the remainder to the second surface, where a second loss by reflection takes place. The fraction of the light transmitted is the ratio of the intensity of the light emerging to the incident intensity. The amount of light energy absorbed is a function of the physicochemical nature and depth of the glass. The losses from reflection depend on the refractive index of the glass. Fresnel showed that the fraction of light energy R lost by reflection from a single *surface* is given by the expression

$$R = (n - 1)^2/(n + 1)^2$$

where n is the index of refraction of the glass in the spectral region being considered. Container glasses have refractive indices of approximately 1.5. Thus R = 0.04 for one surface or 0.08 for two. This means that 8% is lost by reflection, and the maximal transmission is about 92%. Samples of glass can be spectrophotometrically scanned in much the same way as a solution of an organic compound; the procedure is given in the USP. Thus the actual transmission values for a specific piece of glass depend on its thickness and the absorption coefficient of the glass. Figure 5.5 shows spectral transmission curves for various glasses. Regular clear glass excludes only light of $\lambda < 325$ nm, whereas amber glass effectively excludes much of the light of $\lambda < 450$ nm.

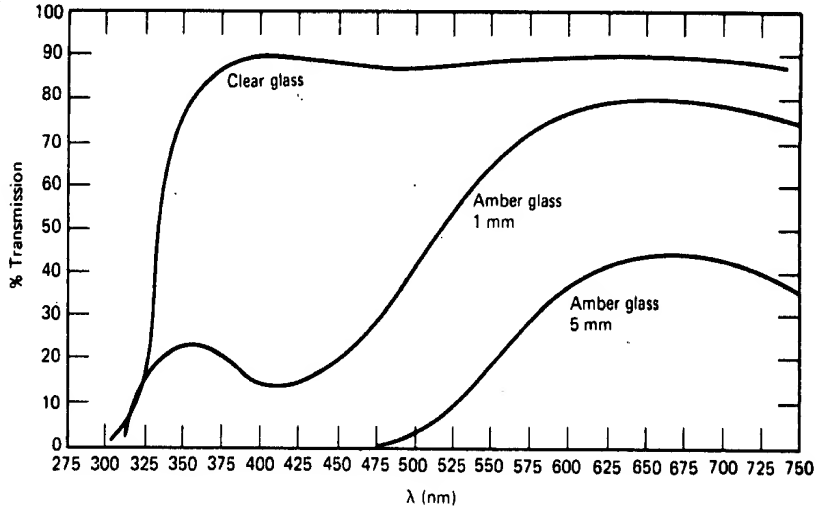


FIGURE 5.5. Spectral transmission curves showing effect of glass type and thickness.

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CHAPTER 6

Solid-State Chemical Decomposition

The chemical decomposition of drugs in the solid state has been the subject of many papers and reviews. However, the mechanism whereby drugs degrade in their pure solid forms is still a matter of debate. The problem is further complicated when the drug is formulated in a complex matrix such as a tablet or capsule; now, not only may the drug itself be intrinsically unstable, but the various excipients (lubricants, fillers, binders, etc.) may act as reactants or catalysts.

In all the earlier chapters, chemical decomposition in solution usually followed pseudo-first-order kinetics. When the same drugs were formulated as suspensions their overall degradation followed pseudo-zero-order kinetics because only that fraction of the drug in solution underwent chemical degradation. In the same way, solid state drug degradation mainly occurs in a solution phase, that is, in solvent layers associated with the solid phase. The source of the solvent for the solid-state decomposition reaction may be

(a) a melt from the drug itself or an ingredient in the formulation that has a low melting point;
(b) residual moisture or solvent from wet granulation;
(c) moisture adsorbed onto excipients such as starch, lactose, or microcrystalline cellulose;
(d) adsorbed atmospheric moisture; or
(e) a solvate or hydrate that loses its "bound" solvent with time or temperature fluctuation.

Since only a fraction of the solid drug is in solution in the tablet, the overall loss of drug often follows pseudo-zero-order kinetics. However, as will

be seen later in this chapter, other factors can lead to the drug loss displaying rather unusual time dependencies.

Chemical decomposition of drugs in the solid state can be divided into these four categories:

(a) ***Solvolysis.*** This is probably the most important reaction for drug degradation in the solid state. As the name suggests, this involves a decomposition by reaction with a solvent. The reaction may be a hydrolysis or other form of degradation discussed in Chapter 4, as well as reactions such as decarboxylation (see *p*-aminosalicylic acid monograph).

(b) ***Oxidation.*** Oxidation involving the interaction of a chemical with oxygen often occurs only in a solvent, although there are examples to indicate that the oxygen may be able to oxidize a drug in the absence of a solvent.

(c) ***Photolysis.*** The photolytic degradation of drugs like the phenothiazines and vitamin A may require the presence of a solvent, but in some cases it may not be necessary to invoke a solvent-dependent reaction. An interesting aspect of this type of reaction is that light penetration into a complex matrix like a tablet or capsule is limited, so that many photolytic reactions are often confined to the surface (<0.3 mm) of a tablet.

(d) ***Pyrolysis.*** This is a thermally induced bond rupture, and it is considered to occur in the absence of solvent. This is not normally an important mechanism for solid-state degradation of pharmaceuticals except where the drug may be exposed in processing to very high temperatures. *p*-Aminosalicylic acid degradation in the 70-80°C range in the absence of moisture may have shown a significant pyrolytic degradation route (1).

A. KINETICS OF SOLID STATE DECOMPOSITION

The loss of drugs by solid-state decomposition does not always follow simple zero-order or first-order kinetics. Figure 6.1 illustrates the overall shape of a plot of percent drug remaining versus time for a hypothetical drug.

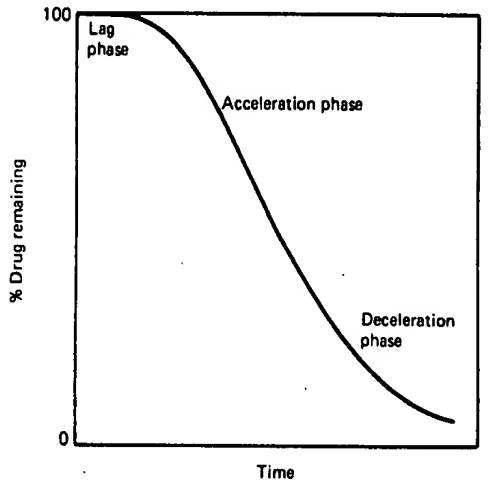


FIGURE 6.1. A hypothetical plot of percentage of drug remaining vs. time for a drug undergoing solid-state decomposition.

Quite often, solid-state degradation curves have a sigmoid shape with a lag phase followed by an acceleration phase. The acceleration phase can be apparent zero order, apparent first order, or higher orders with respect to drug, depending on the conditions of the experiment, such as humidity and temperature, and the mechanism of the degradative process.

Various theories have been put forward to describe the kinetics of thermal decomposition in the solid state (2) in the absence and presence of moisture or of other solvents (3).

Ng has argued that most thermal decompositions can be defined by Eq. (6.1),

$$\frac{d\alpha}{dt} = \bar{k}\,\alpha^{1-p}(1-\alpha)^{1-q} \qquad (6.1)$$

where α is the fractional decomposition, and $\bar{k}$ is a composite rate constant that includes a term for N_0, the number of potential nuclei (decomposition) sites on the solid. The quantities p and q are parameters related to the mechanism of the reaction and generally have the limits of 0-1. The basic idea behind this

equation is that all decomposition occurs initially at nuclei on the crystal surface. These nuclei are stress points, imperfections, or dislocation points in or on the crystal. The rate of decomposition ($d\alpha/dt$) is proportional to α because the actual act of decomposing "induces" an increase in the decomposition rate by causing an increase in the stress or dislocations in the crystal, thereby increasing the number of nuclei. The dependency of the sensitivity of decomposition rate on this increase in crystal stress is defined by p. If $p = 0$, then $\alpha^{1-p} = \alpha$, which says that the rate is directly proportional to α. If $p = 1$, then $\alpha^{1-p} = 1$, which says that the rate is *not* proportional to α. The term $(1 - \alpha)^{1-q}$ is necessary to help describe the sigmoidal shape. It is rationalized by the argument that as the reaction proceeds the dislocations generated will be dependent not only on α but also on $1 - \alpha$; that is, after a certain time the number of dislocations will begin to decrease. Again this decelerating phase will depend on the value of q.

If both p and q are unity, then Eq. (6.1) collapses to Eq. (6.2) and overall zero-order kinetics are observed.

$$\frac{d\alpha}{dt} = \bar{k} \tag{6.2}$$

If $p = 0$ and $q = 1$, then Eq. (6.3) results

$$\frac{d\alpha}{dt} = \bar{k}\alpha \tag{6.3}$$

and the decomposition follows apparent exponential kinetics. If p and q are both equal to zero, then Eq. (6.4) results

$$\frac{d\alpha}{dt} = \bar{k}\alpha(1 - \alpha) \tag{6.4}$$

and the decomposition will follow a sigmoidal shape similar to that illustrated in Figure 6.1. If $0 < p < 1$ and $0 < q < 1$, various other equations evolve (2).

The mathematical concept presented above was developed principally for thermal solid-state decompositions. However, Eq. (6.1) and its variants have also been used empirically to "fit" data under conditions

where the reactions are moisture dependent (4). It could probably "fit" data for almost all solid-state decompositions except those where the decomposition proceeds to some equilibrium state.

The sigmoidal shape seen with solid-state decomposition can also result from an autocatalytic effect of the product of decomposition. This can take two forms. The product of the decomposition may chemically catalyze the decomposition, or the product of the decomposition may be a liquid in which the reactant drug can dissolve and decompose more rapidly. Alternatively the decomposition products may be hygroscopic, causing the surface of the solid to adsorb atmospheric moisture more readily, leading to acceleration of the degradation rate. Other possible reaction schemes could be envisaged to describe a sigmoidal degradation curve.

An apparent zero-order degradation curve, quite often seen for the decomposition of pharmaceutical products, can be rationalized as follows. The drug, drug-excipient blend, or tablet, adsorbs a thin layer of water on the surface of the solid product. The drug dissolves to the extent of its solubility in this water and only the dissolved drug decomposes. This is analogous to drug degradation from suspension, where the observed zero-order degradation rate constant is a product of the intrinsic first-order degradation rate constant in that solvent and the solubility of the drug in the solvent.

B. PHARMACEUTICAL EXAMPLES OF SOLID-STATE DECOMPOSITION

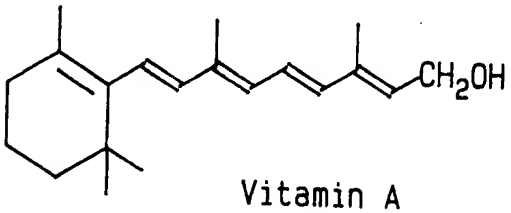
1. Pure Drugs

Before talking about specific examples of solid-state decomposition it is worthwhile mentioning that it is very difficult to design solid-state degradation studies because we may not be aware of all the variables that control solid-state degradation. Also the practical problems of controlling temperature and water vapor pressure above the solid sample, and of guaranteeing a homogeneous sampling of the reaction mixture when the decomposition may be occurring unevenly throughout the sample, can influence the accuracy of

the results.

Based on the nuclei theory of degradation, factors such as particle size of the crystals, how the crystals were formed, how many stresses there were in the crystals, and presence of trace impurities, may all be important variables. Another obvious problem is that polymorphic forms of the drug may degrade differently owing to unlike physicochemical and crystalline properties. All this complicates both the assessment and interpretation of solid-state decomposition results.

Guillory and Higuchi (5) studied the solid state stability of various vitamin A derivatives. The compounds studied were either esters of vitamin A or derivatives of vitamin A aldehyde.



Vitamin A

These authors were interested in the degradation of these compounds in relation to their crystallinity as measured by their melting points. It was argued that the lower-melting-point compounds should decompose more rapidly in the solid state than higher-melting-point compounds of comparable solution stability. The overall loss of vitamin A compounds, maintained at 50°C, followed apparent zero-order kinetics. Table 6.1 is a summary of some of the results.

Overall it can be seen that the lower the melting point the more unstable the compound. It was argued that the degradation probably occurs almost exclusively in a liquid phase on the surface of the crystal and the fraction of drug in the liquid state is related to the melting point of the pure crystalline solid by Eq. (6.5),

$$\log X_1 = \frac{-\Delta H_f}{2.303R}\left(\frac{1}{T} - \frac{1}{T_m}\right) \qquad (6.5)$$

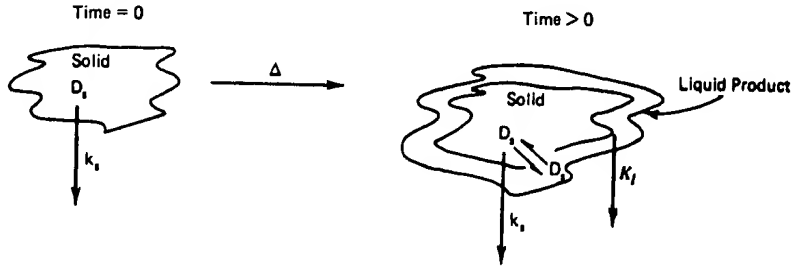
where X_1 is the mole fraction of the component present as a melt, or the amount of liquid phase present, ΔH_f is the molar heat of fusion, R is the gas constant, T_m

TABLE 6.1. Table Comparing the Apparent Zero-Order Degradation Rates of Various Vitamin A Derivatives at 50°C with Their Melting Points

Derivative of Vitamin A	10^2 k (mol/h)	Melting Point, (°C)
Acetate	27	57-58
Phthalimide-N-acetate	4.8	111-112
Nicotinate	2.5	93-94
3,4,5-Trimethoxybenzoate	1.4	85-86
Succinate triphenyl-guanidine salt	0.76	140-140.5
Benzhydrazone	0.38	181-182

and T are the melting point of the pure solid and the temperature of the study, respectively, in degrees Kelvin. It was proposed that the rate of reaction will be proportional to X_1, therefore a linear relationship should be seen between log k and $T^{-1}{}_m$. Such a relationship is approximately adhered to in this case.

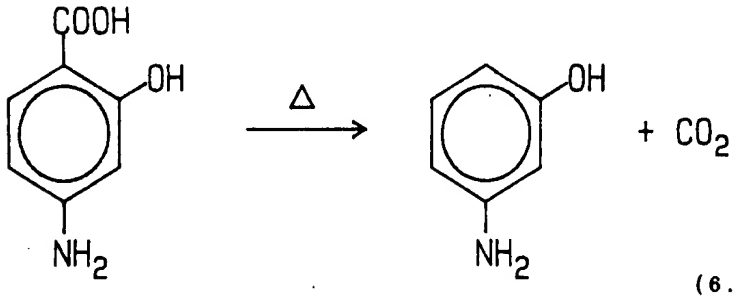
These findings were supported by other studies. In particular, Carstensen and Musa (6) studied a series of substituted benzoic acids that decomposed by decarboxylation to produce a liquid (the decarboxylated product) and a gas (carbon dioxide). This process can be illustrated by Scheme 6.I, where k_s is the decomposition rate in the solid and k_l the decomposition rate in the liquid.



SCHEME 6.I

By fitting their data to a mathematical model consistent with Scheme 6.I, Carstensen and Musa found that the Guillory-Higuchi relation was followed, that is, a plot of log k_s vs. T^{-1}_m was linear.

p-Aminosalicylic acid (PAS) decarboxylation, Eq. (6.6),



(6.6)

occurs when PAS is exposed to elevated temperature both in the absence and presence of water vapor. The loss of PAS in the solid state is illustrated in Figure 6.2. Note that there is a lag time, which is inversely related to temperature, and an acceleration

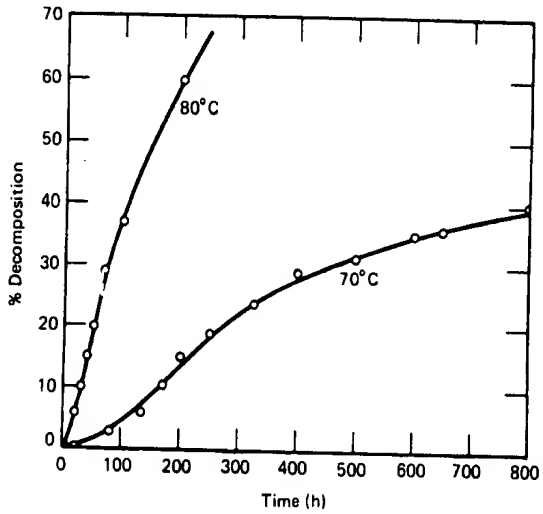
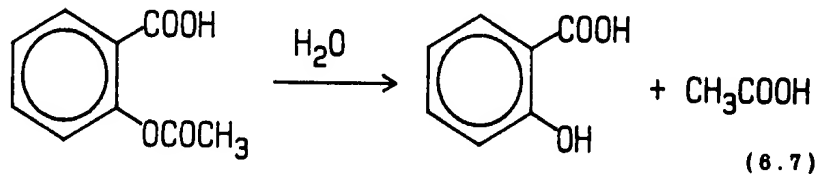


FIGURE 6.2. Plot of percentage decomposition vs. time for the thermal solid-state decarboxylation of PAS at 70 and 80°C. (Data taken from Ref. 1.)

phase, the rate of which increases with increasing temperature (1). Kornblum and Sciarrone (1) studied the effect of pressure and particle size as well as moisture on this reaction. Treating the acceleration phase as a zero-order portion, they observed that a plot of log k vs. 1/T was linear, that is, it displayed apparent Arrhenius behavior, with an apparent activation energy of 41 kcal/mol. In the presence of moisture, the lag phase was shortened and PAS decomposed more rapidly. The data of Kornblum and Sciarrone, as well as visual observations of the crystals cracking along stress lines, supported the idea of a nuclei model for the decomposition.

The importance of moisture in promoting solid-state decomposition of various pharmaceuticals cannot be overemphasized. Moisture is considered to be sorbed as a thin film on the surface of the solid. The results of Kornblum and Sciarrone with PAS were confirmed by Carstensen and Pothisiri (7), who also studied PAS decarboxylation in the presence of moisture. They proposed that the volume of adsorbed moisture was proportional to water vapor pressure above the solid. The drug is proposed to degrade in this thin film of moisture. Leeson and Mattocks (8), in their classic study of aspirin hydrolysis, proposed a very similar treatment for their data. The effect of moisture and temperature on the degradation of acetylsalicylic acid (aspirin) to salicylic and acetic acids, Eq. (6.7), is shown in Figure 6.3.



(6.7)

The sigmoid shape described earlier is particularly obvious at the higher temperature and higher water vapor pressures.

Leeson and Mattocks (8) and others (9) proposed that the acceleration phase of aspirin degradation could be accounted for by the reasonable assumption that the products of the degradation, acetic acid and salicylic acid, lowered the pH of the sorbed moisture layer, thus further catalyzing aspirin degradation

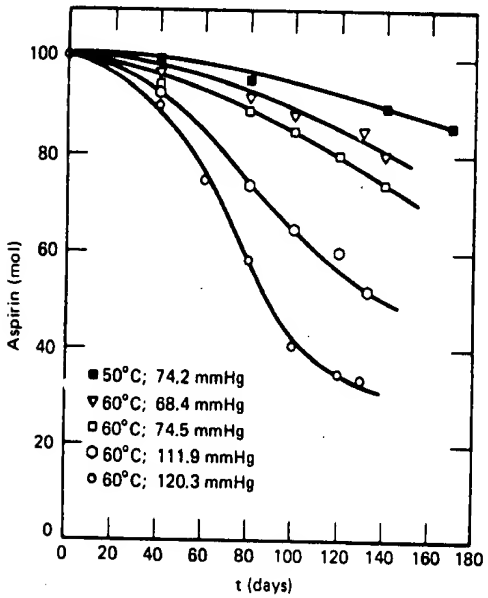


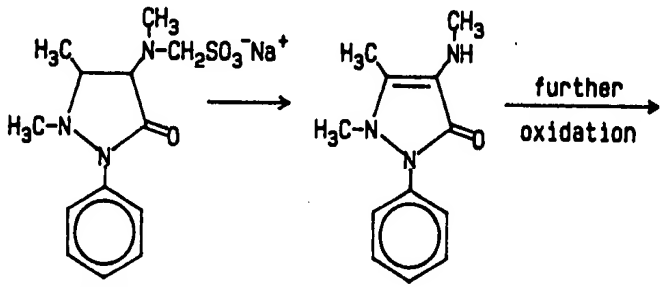
FIGURE 6.3. Plot of mol% aspirin remaining as a function of time, in days, for the solid-state decomposition of aspirin maintained at 50 and 60°C and varying aqueous pressures, in mm Hg. [Taken from Leeson and Mattocks (8).]

since aspirin is subject to acid catalysis at low pH (see Fig. 1 in aspirin monograph). Although equations were derived that appeared to describe the data adequately (8,9), Hasegawa et al. (10) and Okamura et al. (4) have challenged the model experimentally. They studied the degradation of aspirin and some of its analogs under various moisture and temperature conditions to the point where the decomposition curves were in the middle of the acceleration phase. They then removed the products of the degradation (the acetic and salicylic acids) by subjecting the solid to high vacuum. After the solid was placed back in the controlled temperature and humidity environment, the degradation of the aspirin and its analogs proceeded as if nothing had changed. These authors proposed an empirical nuclei model to fit their data that is actually just a specialized form of the equation proposed by Ng (2), Eq. (6.1).

A particularly interesting observation was made by Okamura et al. (4) when they isolated two types of crystals of 5-nitroacetylsalicylic acid (4-nitroaspirin) from a recrystallized batch of material. One was called "column shaped" and the other "ramified." The ramified or less perfect crystals degraded significantly faster than the more uniform column crystals, and the product of the degradation, 4-nitrosalicylic acid, observable under a polarized microscope as dark regions, appeared as "dark spots" on the surface of the column-shaped crystals but were concentrated at the forks of ramified crystals (4). The higher reactivity in apparent areas of stress in the crystals was consistent with the nuclei model discussed earlier.

Numerous studies with other pharmaceuticals have confirmed the importance of temperature and humidity as two of the most important variables that significantly affect the chemical stability of drugs in the solid state.

Although it is obvious how moisture affects hydrolytic mechanisms, it is less obvious that moisture is often an important variable in oxidative and photochemical solid-state degradations. In the case of oxidative cleavage, the reactions often occur in solvent layers on the surface of the solid or at nuclei sites. Molecular oxygen dissolved in such solvent sites is responsible for the cleavage. There are reactions in which molecular oxygen could be envisaged as penetrating the crystal lattice to promote a true "drug" solid degradation, but the majority of cases are more like a recently studied example (11) involving the oxidation of sulpyrine, Eq. (6.8).



sulpyrine (6.8)

It was found that the sulpyrine solid-state degradation rate was proportional to water content and to the partial pressure of oxygen over the solid. Without added water, but in various controlled humidity chambers, high humidity dramatically promoted the breakdown.

The case of sulpyrine brings up an interesting point. As can be seen from its structure, sulpyrine has considerable aqueous solubility as a sodium salt of a weak acid. Therefore its ability to dissolve and react in an adsorbed moisture layer will be significant. A salt of sulpyrine having lower aqueous solubility would probably display improved stability characteristics if only that fraction of drug in solution is capable of degrading. Of course, the improved stability that might be seen with a poorly water soluble salt might be counterbalanced by the diminished bioavailability from a dosage form owing to the altered physicochemical properties of the drug.

Solid-state photolytic degradation problems are generally easily solved by excluding light from the solid. Photolytic reactions in the solid state are self-limiting in that light penetration into a solid mass is often limited by depth of penetration. Light striking the surface of a solid mass is absorbed, scattered, or reflected. Penetration to the extent of 0.3 mm in a tablet has been observed by recording the fading of dyes incorporated into a tablet. For such tablets a line of demarcation can often be observed if a cross-sectional cut of the tablet is made.

2. Drug-Excipient and Drug-Drug Interactions in Solid Dosage Forms

As mentioned earlier, solid-state decomposition mechanisms are not as well understood as those involving solutions. When this is further complicated by incorporating the drug into a solid matrix such as a tablet or capsule with other ingredients like fillers, lubricants, and binders, it is difficult to interpret the data with a high degree of confidence. An added complication is seen with solid dosage forms like lyophilized products for parenteral administration. Here the drug entity often forms an amorphous powder with the other ingredients of the dosage form, such as buffers and caking agents. The loss of crystallinity and residual moisture can play havoc with the stability (12).

Temperature effects on solid dosage form stability studies can be complicated when:

(a) humidity is not simultaneously controlled;
(b) one of the ingredients, either the drug or an excipient, has a low melting point;
(c) one of the ingredients has loosely bound water, and alterations in temperature change the degree of binding of the water to the excipient;
(d) one of the ingredients of the dosage form is present as a hydrate or solvate and is capable of giving up this bound solvent to an unbound state as a result of temperature changes; or
(e) the solid dosage form is stored in different types of containers, open or closed, permeable or hermetic, and so on, which may affect the stability in different ways.

If the loss of drug from a particular dosage form follows a recognizable pattern that is interpretable mathematically, the parameters used to define drug loss with time can be compared as the temperature is changed. This comparison usually takes the form of an Arrhenius plot, the logarithm of the parameter being plotted against the reciprocal of the absolute temperatures. If Arrhenius behavior is followed an "apparent" energy of activation is calculated. Adherence to such behavior allows the pharmaceutical scientist to estimate the stability of the drug in that dosage form at some other temperature, but the assumption must be made that the mechanism operative at the experimental temperatures is the same as at the extrapolated temperature (most often room temperature). The word "apparent" is used here because more factors affect the magnitude of this activation energy than simply the effect of the temperature on the rate-controlling chemical reaction. Temperature affects the solubility of the drug in a solvent layer (increasing degradation), it increases the intrinsic rate of reaction (increasing degradation), and it may alter the availability of solvent in which the reaction occurs. This last effect increases degradation if the amount of solvent available for reaction increases with increasing temperature, that is, if "bound" solvent in the

form of a hydrate or solvate is released, but it may decrease degradation if the higher temperature actually helps drive off loosely bound water in the tablet, thus decreasing the amount of solvent present. This might happen if the study were undertaken in an environment where the water vapor pressure above the dosage form is low or negligible.

The effect that moisture or water content of the dosage form has on stability is well documented. For the most part, increasing moisture exposure, whether from one of the dosage form excipients or by atmospheric equilibration, is deleterious to the chemical stability of drug molecules. The moisture content of some commonly used tablet excipients is given in Table 6.2.

TABLE 6.2. Moisture Content of Some Common Tableting Excipients at 25°C, Stored at Different Relative Humidities (RH)[a]

Excipient	Percent Equilibrium Moisture Content at 25°C		
	33% RH	75% RH	100% RH
Dicalcium phosphate, anhydrous USP	<0.1	<0.1	7.0
Lactose USP, spray dried	0.5	1.0	21.5
Magnesium stearate	3.1	3.5	--
Cellulose, microcrystalline N.F.	3.7	8.1	--
Polyethylene glycol 3350 N.F.	<0.3	2.0	62.2
Pregelatinized starch N.F.	7.8	14.7	36.4
Starch USP corn	8.0	14.4	16.5
Povidone USP	12.2	27.8	--

[a]Equilibrium moisture content from selected excipients reported by Callahan et al. (13).

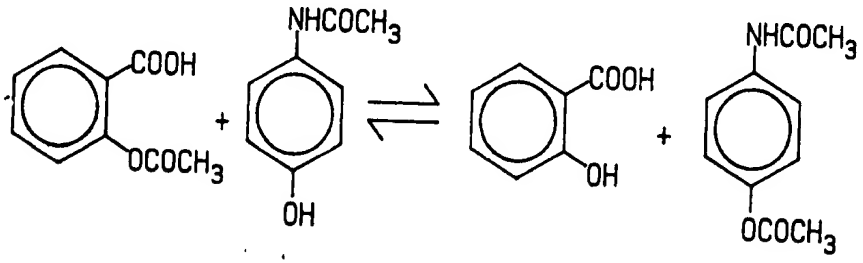
As can be seen, most excipients have an appreciable water content. Whether all this moisture is available to the drug will depend on whether the moisture is loosely or strongly bound and whether even the loosely bound moisture can come in contact with the drug. For example, Jain et al. (14), in studying the stability of captopril as the pure material and in the presence of various tableting excipients, found that captopril itself (pure) was stable to temperature and high humidity but in the presence of excipients was readily oxidized. As expected, the degradation was accelerated by high humidity, temperature increases, and oxygen concentration. Starch, which has a fairly high moisture content when compared with lactose and microcrystalline cellulose, caused minimal degradation. Consistent with this was the observation that a reduction in particle size of either the drug or the excipient slowed the rate of degradation. It seems reasonable that particle-to-particle interaction (packing geometry) may play a very important role. With other drugs contrary observations have been made, that is, there does not appear to be any simple explanation or generalization for the overall stability behavior of solid-state degradation and the moisture content of excipients.

Excipients can also affect the stability of drugs by: (1) acting as surface catalysts; (2) altering the pH of the moisture layer; and (3) undergoing direct chemical reaction with the drug. For example, Carstensen et al. (15) studied the stability of thiamine in the presence of microcrystalline cellulose as a function of added moisture. The presence of a small amount of added moisture accelerated thiamine decomposition. However, as greater amounts of moisture were added the rate of degradation went through a maximum at about 6% water. This is consistent with the idea that the first adsorbed moisture layer is more reactive towards the drug. In fact thiamine was quite stable in an aqueous slurry of microcrystalline cellulose.

Excipients that on partial dissolution in an adsorbed moisture layer may change the local pH to a level where it can be deleterious to drug stability will consequently accelerate the degradation of the drug. On the other hand, an excipient may stabilize the drug if it maintains a favorable local pH (within boundaries) for optimal stability. Quite often measurement of the pH of a slurry of a possible tablet formulation can indicate whether stability could be a problem. Walters (16) recently showed, for diethyl-

propion hydrochloride tablets, that the stability of the active drug correlated well with the pH of an aqueous tablet slurry. Diethylpropion hydrochloride is quite stable at acidic pH and very unstable at neutral to alkaline pHs. Tablet formulations that generated slurries with acidic pHs (pH 2.4-3.5) were stable, whereas slurries with pHs > 4 were quite unstable.

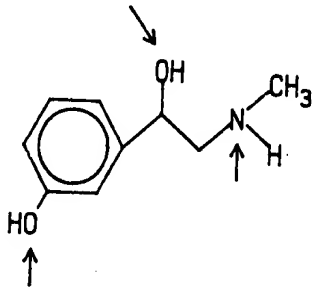
Direct chemical reaction between a drug and an excipient is not a commonly reported or well-documented problem. Some classical examples of drug-drug interactions have, however, been noted. The majority of reported examples involve the reaction of aspirin with hydroxyl- or amine-containing drugs through a transacylation reaction. The high incidence of aspirin/other drug interactions results in part from the use of aspirin in many combination products, especially for the relief of pain, cold, and flu symptoms. Aspirin has been shown to react with codeine and with acetaminophen (Eq. 6.9) to form their corresponding acetyl esters (17,18) and salicylic acid.



(6.9)

Similar results were seen for the reaction of aspirin with *p*-hydroxyamphetamine (19). Note that the reaction defined by Eq. (6.9) is presented as an equilibrium reaction rather than a unidirectional reaction. Obviously, as O-acetylacetaminophen accumulates it could react with the formed salicylic acid to produce aspirin and acetaminophen. Exactly where this equilibrium lies will depend on the relative acetylating ability of aspirin and O-acetylacetaminophen and the nucleophilic reactivity of the phenolic hydroxyl groups of salicylic acid and acetaminophen.

The most commonly reported drug-drug interaction involves the reaction of aspirin with phenylephrine to form the mono-, di-, or triacetylphenylephrine depending on reaction conditions.



(arrows indicate potentially reactive sites)

In practice this problem of transacylation reaction between an active acyl donor (e.g., aspirin) and an acyl acceptor can be prevented by the physical separation of the reactive species. In the case of commonly used cold remedies containing aspirin and phenylephrine this is achieved by the use of layered tablets. For example, a formula for a commonly used cold preparation, Dristan®, is

R_x:

aspirin,
phenylephrine HCl,
phenindiamine HCl,
caffeine,
aluminum hydroxide and magnesium carbonate in a co-dried gel

The tablet is a yellow and white layered tablet where presumably the reactive species are in separate layers and the only contact between the reactive ingredients is at the interface of the two layers.

Another interesting drug-drug interaction is seen in propoxyphene compound preparations. The degradation of aspirin to salicylic acid is accelerated by propoxyphene hydrochloride. Goldberg and Nightingale (20) showed that this degradation was very sensitive to formulation variables when preparations from three manufacturers were compared. It was interesting to note that the aspirin in one brand was particularly stable, apparently because propoxyphene is physically

separated from the aspirin through incorporation in a pelleted form (20). From structural considerations, it is hard to imagine a mechanism whereby propoxyphene accelerates the degradation of aspirin.

C. METHODS OF STABILIZATION

There are a number of ways to inhibit solid-state drug degradation. One method involves altering the properties of the drug. As has been emphasized in this as well as in the earlier chapters, solvolytic reactions can be prevented by physically separating two reactive species. In the case of solvolytic reactions and especially hydrolytic reactions this involves minimizing the contact between the drug and water. Since the drug must dissolve in what little moisture is present in the formulation, the degradation rate will be a function of the solubility properties of the drug in water. Obviously drugs that are hygroscopic and that degrade in the presence of moisture are going to give rise to stability problems. Converting the drug into a nonhygroscopic form through crystal modification or by preparing a less-water-soluble salt may solve the stability problem. The apparent zero-order degradation rates of drugs in the solid state can often be defined by Eq. (6.10),

$$-\frac{d(\text{Drug})}{dt} \propto k(\text{amount of dissolved Drug}) \qquad (6.10)$$

$$\text{Amount of dissolved Drug} = (\text{volume of available solvent}) \times (\text{saturated solubility of the Drug}) \qquad (6.11)$$

assuming that degradation only occurs in the dissolved fraction of drug.

As can be seen with Eqs. (6.10) and (6.11), the degradation is a function of both the solubility of the drug and the amount of available solvent. Therefore a second method of stabilization would be to minimize the amount of moisture in the formulation by the judicious choice of excipients, manufacturing conditions, storage conditions, and packaging of the final and intermediate products.

If the degradation is sensitive to the pH of the moisture layer, this property possibly can be adjusted by the inclusion of buffers in the formulation, as long as the presence of the buffers does not lead to other problems such as an incompatibility with other excipients or added moisture in the form of hydrates.

If the degradation of the drug is sensitive to oxygen or light, the influence of these variables can be minimized by their exclusion through dosage form coatings and by proper packaging.

Unfortunately, our knowledge of solid-state degradation mechanisms, especially in complex matrices such as tablets and capsules, is not at the same level of development as that for solutions. We do not have a good understanding of all the critical variables that appear to be contributing to the degradation mechanism. Identifying the important variables other than temperature and humidity should be objectives of future systematic studies of solid state decomposition.

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CHAPTER 7

Strategy and Tactics of Stability Testing

The study of drug-decomposition kinetics, the development of stable dosage forms, and the establishment of expiration dates for commercially available drug products are activities carried out mainly in the laboratories of the pharmaceutical industry. The practicing pharmacist may, however, be interested in the approaches taken to study drug stability, and this chapter briefly describes the requirements and methods of a stability-testing program.

The use of kinetic and predictive studies for establishing credible expiration dates for pharmaceutical products is a relatively recent phenomenon. Prior to 1950 only qualitative and semiquantitative methods were used. The trend now is toward rigorous, scientifically designed studies using reliable and specific assays, and appropriate statistical concepts.

Stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications. Assurances that the packaged product will be stable for its anticipated shelf life must come from an accumulation of data on the packaged drug product. This process begins at the early development phases and continues through monitoring of the marketed batches. Through this process, varying objectives must be reflected in the design of different stability studies. All studies have common elements, including general design, analytical method, storage conditions, testing schedules, samples, lots and containers, and data evaluation. This chapter is

[1]Prepared with the assistance of Pradip K. Banerjee.